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GROWTH HORMONES IN PLANTS

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GROWTH HORMONES IN PLANTS

AUTHORIZED ENGLISH TRANSLATION OF

DIE WUCHSSTOFFTHEORIE

UND IHRE BEDEUTUNG FÜR DIE ANALYSE DES WACHSTUMS

UND DER WACHSTUMSBEWEGUNGEN DER PFLANZEN

BY

P. BOYSEN JENSEN

Professor of Plant Physiology, University of Copenhagen

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with the Collaboration of

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Department of Botany, Connecticut College

Expanded to include 188 new contributions to the literature and 40 additional illustrations

FIRST EDITION

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FOREWORD

It is with the greatest satisfaction that I follow the increasing interest in America and England in the investigations concerning growth substances in plants; indeed, a series of important contributions to our knowledge about these substances originate from laboratories in these countries. It is, therefore, a great delight for me to have American friends translate my book "Die Wuchsstofftheorie" into English. I want to express my most sincere thanks to Professor George S. Avery, Jr., Dr. Paul R. Burkholder, Dr. Harriet B. Creighton, and Miss Beatrice A. Scheer, who have undertaken the troublesome and ungrateful work, and who have likewise revised the text and brought it up to date. I hope that the translation and revision may make the book more easily available to American and English colleagues and that it may also stimulate new studies on these promising problems, as there are still so many points needing further investigation.

P. Boysen Jensen.

Copenhagen,
June, 1936.

PREFACE

It is with enthusiasm that we bring a translation and revision of Professor Boysen Jensen's "Die Wuchsstofftheorie" to botanists in English-speaking countries. It is the first comprehensive review of the literature dealing with the role of growth hormones in normal growth and tropisms of plants. In translating and revising the book to include the 1935 literature, it is our hope to stimulate sound progress in this important new field of plant physiology.

Plant growth hormones have been recognized by many investigators as offering fruitful opportunities for study, and there is ample evidence in the contributions of the past year or two that research in this field is progressing at a rapid pace. One discovers in the literature that growth hormones promote cell enlargement in shoots of higher plants, initiate the development of roots (but at the same time inhibit their growth in length), inhibit the development of lateral buds, stimulate cell division in the cambium, and bring about callus formation. Such diverse effects are of great interest, but in many instances more evidence must be obtained to prove the theoretical views which we now possess. Professor Boysen Jensen's review of the subject makes clear numerous weaknesses and gaps in our knowledge and thus helps point the way for future research.

The arrangement of certain chapters has been modified in the translation so that students not already acquainted with the literature may become familiar with techniques and general methods of procedure. Certain parts of the book have been condensed slightly, while others have been expanded to include data from the approximately 200 new citations added to the bibliography. The essential features of the controversial final chapter of the German edition have been included so far as possible in earlier chapters. In an attempt to make the American edition as useful as possible to students, we have added an index, a summary at the end of each chapter, and numerous illustrations. The following new figures have been inserted: 7, 8, 13–15, 17–20,

23–26, 28–31, 33–44, 50, 55, 58–62; and Figs. 21 and 22 have been substituted for Figs. 10 and 11 of the German edition. The historical development of the subject has been presented in a series of diagrams (Figs. 1 and 2). Plant growth substances, that is, *Wuchsstoffe*, have been referred to by various workers as growth hormones, growth regulators, growth enzymes, phytohormones, and auxins. We have used all of these terms. A really satisfactory terminology, based on the chemical nature of the compounds in question, will have to wait until more is known.

A selected list of titles dealing with the influence on plants of substances such as bios, folliculin, and other sex hormones, those affecting the growth of fungi, etc., has been added for the convenience of students interested in these topics. They are not discussed in the text.

It is a pleasure to express our appreciation of Professor Boysen Jensen's interest and cooperation in the effort to make this book available to English-speaking botanists.

George S. Avery, Jr.
Paul R. Burkholder.

Connecticut College,
June, 1936.

PREFACE TO THE GERMAN EDITION

Twenty-five years have passed since the discovery of growth substance in the Avena coleoptile. The development of growth-substance research in this period of time is characterized by a continually growing mass of publications. The growth-substance literature, in a narrow sense, includes at present about 200 individual papers. Investigations in this field are appearing continually, so that important new discoveries must be taken into consideration each year. One can foresee great forward strides during the next few years in this line of study.

What has been accomplished so far by this extensive activity? The most important contributions are as follows: The significance of growth substance for photo- and geotropic curvatures was demonstrated first in the Avena coleoptile. Then its effect on normal growth was investigated, and it was shown that growth substance plays an important part in the positive geotropic curvature of the main root. Next the general occurrence of growth substances was demonstrated in higher and lower plants and in animals. Finally it became possible to prepare growth substance in a pure state. The main result has been a glimpse into the mechanism of the orientation of higher plants in space.

Do the results that have been obtained justify the effort—has growth-substance research had its reward? Perhaps such a question should not be asked. I feel that it would be unfair to suppress the fact that, although great advances have been made, we are still far from an explanation of photo- and geotropic processes. It is satisfying to know that something has been attained that may be regarded as progress in our knowledge of life processes. One must become reconciled to the fact that in advanced physiological research, problems become so involved that every stride forward requires a great output of labor.

The author has attempted to present the chief lines of investigation and the main results of research done in the last twenty-

five years. He has endeavored to present objectively the views of various investigators, to distinguish as clearly as possible between experimentally founded fact and more or less hypothetical views. Particular attention is given to the fundamentals of the growth-substance theory—and the author hopes, therefore, that his work will be useful to those carrying on growth-substance research.

P. Boysen Jensen.

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GROWTH HORMONES IN PLANTS

CHAPTER I

INTRODUCTION AND HISTORICAL SKETCH

GROWTH AND GROWTH SUBSTANCES

The growth of living organisms is of fundamental importance to all students of biology. It implies a permanent increase in the size of the whole organism or its parts as a result of the incorporation of materials from the environment. Growth is due chiefly to the absorption of water, the synthesis of new protoplasm, extension of cellular boundaries, and increase in weight. As an organism grows, it becomes differentiated into parts that perform specific functions.

Growth in plants results from the integration of many internal processes. In an attempt to analyze the discrete chemical reactions and physical conditions that contribute to growth, it may be useful to classify the substances concerned into two groups: nutritional substances and regulating substances. In the first group, considered in the broadest sense, belong water, minerals, gases, and the organic foodstuffs which supply energy for building the plant's structures. Following Huxley's suggestion (1935), the second group of regulating substances may be conveniently subdivided as follows: (1) localized chemical activators whose range of influence may be limited strictly to intracellular activities (*e.g.*, those concerned with various genic effects) or to a comparatively small sphere; (2) hormones which exercise specific effects upon cells or tissues other than those by which they are produced. To the latter group of substances belong those growth-regulating materials which are the subject of this book.

The significance of chemical correlation in plant physiology and morphogenesis has captured the imagination of botanists ever since the discovery in plants of chemical substances which might be called hormones. The word *hormone*, derived from the Greek ὁρμάω and meaning "I arouse to activity," was suggested by Hardy and first applied in animal physiology by Starling (1906) in discussing the substance secretin; it was later defined by Starling (1914) as "any substance normally produced in the cells of some part of the body and carried to distant parts which it affects for the good of the body as a whole." It has been shown (Huxley, 1935) that all gradations exist between hormones and local activating substances and between the latter and ordinary by-products of metabolism which are less specific in regard to the nature of the structures acted upon. The word *hormone* was used for the first time in connection with plants by Fitting (1910), who found that a substance present in orchid pollen caused swelling of the gynostemium in the orchid flower.

In dealing with the phenomena of growth, careful distinction has not always been maintained in the past between the substances which may be correctly termed hormones and certain other types of materials. In comparatively recent years, a number of terms, more or less useful but not particularly well-defined, have been proposed to designate newly discovered functional materials, such as hormone, enzyme, vitamin, bios, Wuchsstoff, etc. Although these terms may overlap in meaning, they are temporarily useful until more information is available. The definition of a hormone is given above in biological terms because hormones play a role only in living organisms; they are chemical substances which have a specific influence on correlation and differentiation of the organism. They are effective when present in minute amounts and control growth in plants in some way other than by direct nutritive means. The substances influencing growth through direct nutritive effects include *vitamins* (accessory or protective food factors in animals), *bios* (substances that apparently function much like vitamins in the growth of certain plants—Miller, 1930; Kögl, 1935, Mitt. XIV), etc. *Enzymes* are produced also by living organisms and promote chemical reactions either within or outside the organism and are not used up. In some instances a given substance may fall into more than one category. Plant-growth

substances, *i.e.*, *Wuchsstoffe*[1], have been referred to by various workers as growth hormones, growth regulators, growth enzymes, phytohormones, and auxins. They include compounds that promote the growth of the Avena coleoptile and the hypocotyls, stems, and leaves of various dicotyledonous plants, but they apparently retard the growth of roots. They are known to be produced by Avena, Zea, Rhizopus, Aspergillus, various bacteria, and numerous other organisms. They are ether-soluble, sensitive to peroxide, and have an acid character.

HISTORICAL SKETCH

Botanists first became acquainted with growth substances through studies on tropisms, *i.e.*, those growth curvatures that take place in response to unilateral stimulation of an organ by light or its displacement from the usual position of equilibrium with respect to the force of gravity, etc. A short historical survey of the earlier contributions to our knowledge of the growth phenomena concerned in photo- and geotropism is presented here as a background for the information that will follow.

Darwin.—In a book entitled "The Power of Movement in Plants," Darwin (1881) recorded extremely valuable experiments and reflections upon the movements of plants in response to light. Among other things, he demonstrated a localization of the phototropic stimulus in certain plants. The main object on which he experimented was the coleoptile of *Phalaris canariensis*. When this organ was unilaterally illuminated, a strong positive phototropic curvature resulted. If the tip of the coleoptile was darkened by a tinfoil cap or a darkened glass cap, and only the lower part was unilaterally illuminated, curvature usually did not result. However, if the procedure was reversed, *i.e.*, if the upper part of the coleoptile was unilaterally illuminated while the lower part was darkened (by means of moist sand), a phototropic curvature took place in the lower portion (Fig. 1). It was shown also that a coleoptile does not react phototropically when 2.5 to 4 mm. of the tip is removed (Fig. 1). Darwin concluded (1881, p. 474) "that when seedlings are freely exposed to a lateral light, some influence is transmitted from the upper to the lower part, causing the latter to bend." Localized sensitivity to light and conduction of a stimulus were observed also in the coleoptile

[1] This refers to *Wuchsstoffe* A. The term *Wuchsstoffe* B refers to a different class of substances, such as Bios (see Nielsen, etc., Supplementary Bibliography).

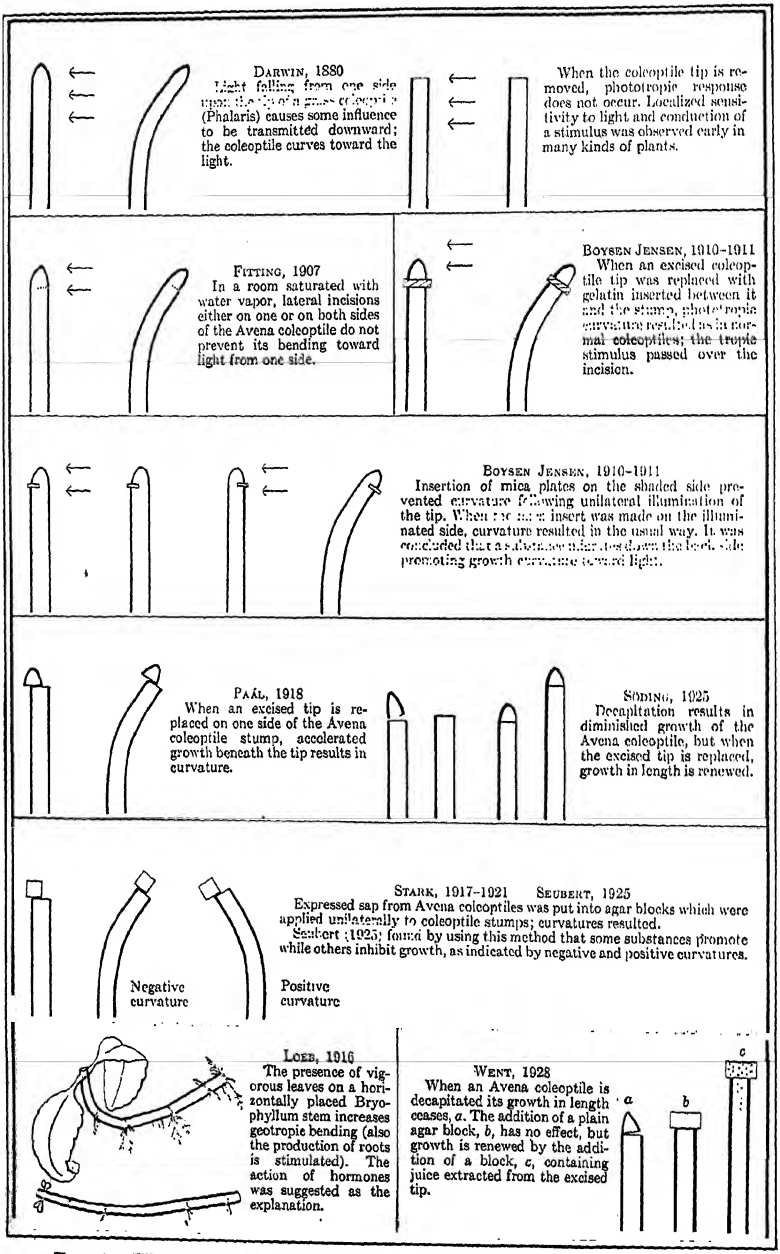


FIG. 1.—Historical outline of the early discoveries concerning plant growth

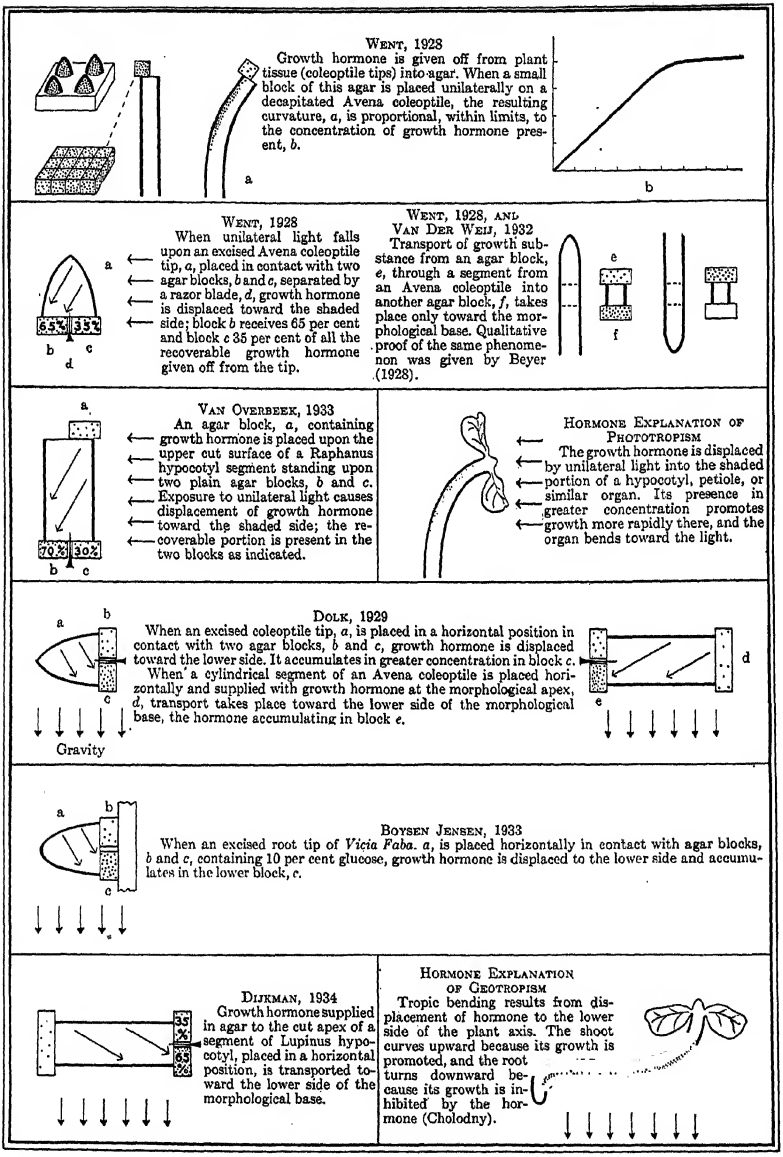


FIG. 2.—Outline of recent contributions to our knowledge of plant growth hormones.

of *Avena sativa*, in the hypocotyls of *Brassica oleracea* and *Beta vulgaris*, and in the negatively phototropic movements of roots.

Wiesner.—Although the significance of Darwin's investigations was recognized fully in the first edition of Pfeffer's "Plant Physiology," the conclusions were criticized adversely by Wiesner (1881).

This criticism is of only historical interest, but it should be mentioned briefly. The curvature that occurs in the lower part of the hypocotyl of *Brassica oleracea* when the tip is illuminated was explained by a factor that Wiesner called a "traction growth" (*Zugwachstum*). When the upper part curved under the influence of unilateral light, its weight supposedly had a unilateral effect upon the lower part. The extended shaded side was interpreted as growing more rapidly than the compressed front side.

This point of view does not seem at all convincing; moreover, only a few experiments were performed with grass coleoptiles. Yet, doubt was thrown upon the correctness of Darwin's conclusions. At the suggestion of Pfeffer, Rothert (1894) investigated the problem of phototropic stimulus conduction in a very thorough manner at the Leipzig laboratory.

Rothert.—The outcome of Rothert's work was a complete confirmation of that of Darwin. Conduction of the phototropic stimulus was demonstrated in a series of different plant organs, including coleoptiles of grasses, seedling axes of numerous dicotyledons, orthotropic leaves, petioles, etc. It developed, however, that the localization of phototropic sensitivity was not actually so marked as had been supposed from Darwin's experiments. Darwin had maintained that only the tip of the Avena coleoptile is phototropically sensitive; Rothert showed that a weak phototropic curvature resulted from unilateral illumination of the basal part. It was found also that sensitivity and mobility are very sharply separated in the seedlings of some grasses. In certain cases only the coleoptile was sensitive to light, and the curvature took place in the first internode below the coleoptile where sensitivity was completely lacking.

With regard to the paths of stimulus conduction in the Avena coleoptile, Rothert noted the following: In the coleoptile cylinder two vascular bundles are situated opposite one another, not joined by cross-connecting bundles (Fig. 21). In an attempt to

determine whether or not these play an essential role in stimulus conduction, Rothert proceeded as follows: Both bundles were severed; the tip was exposed to light; and the lower wounded portion was darkened. A phototropic curvature occurred in this basal portion, and from this Rothert concluded that "it is proved that the heliotropic (phototropic) stimulus is conducted in the parenchyma of the fundamental tissue."

Fitting.—Carrying the work of Rothert further, Fitting (1905–1906, 1907) studied the fundamental processes of stimulus conduction. The question that he set out to investigate was stated as follows: "In tropism, how is the organ of perception so linked with the zone of reaction that the externally applied stimulus can indirectly determine the direction of the curvature?" To solve this problem, Fitting tried to ascertain whether the conduction of a stimulus on a certain side of the coleoptile is in any way oriented with respect to the direction of light. The experiments, on Avena coleoptiles for the most part, were carried out in a room saturated with moisture at a temperature of 30°C. The influence of incisions upon growth and curvature of the Avena coleoptile was studied first. It was found that the growth rate remained practically unchanged by unilateral wounding. Weak curvatures of the coleoptiles were observed, first away from the wound and then toward it. The influence of unilateral transverse incisions upon longitudinal conduction of a stimulus in the Avena coleoptile was then investigated. Incisions were made midway between the base and the tip in coleoptiles measuring 1 to 1.5 cm. in length. The coleoptiles were darkened past the point of incision with tinfoil tubes or with collars made of black paper. Then, if the tip of the coleoptile was illuminated unilaterally, a decided positive phototropic curvature resulted in the darkened basal portion under the incision, no matter what the orientation of the incision was with respect to the direction of light, *i.e.*, whether the incision was on the illuminated or on the shaded side (Fig. 1). These experiments were modified in various ways, but the result was always the same. Even when two incisions were made, one above the other and on opposite sides, the conduction of the phototropic stimulus met with no interference.

Fitting concluded that the stimulus was conducted around an incision and transmitted exclusively through the living material.

When two incisions were made one above the other, he thought that the stimulus conduction was not disturbed by the insertion of tinfoil, no matter how oriented with respect to the direction of light. However, if there were two transverse incisions with tinfoil or mica inserts one above the other, the phototropic curvature under the incisions was very slight. Fitting surmised that the absence of curvature in the latter case was due to drying out of the tip, since the vascular bundles had been severed.

The phototropic curvature in the Avena coleoptile is caused by a difference in the rate of growth of the two sides, the darkened side growing more rapidly than the illuminated. Fitting observed that a positive phototropic curvature resulted whether an incision was made upon the lighted (front) or upon the shaded (back) side. This meant that if stimulus conduction occurred only in living tissue, it must take place in the former case on the back side and in the latter on the front side. The final result of stimulus conduction is the same in both cases; *i.e.*, the shaded side of the Avena coleoptile grows more rapidly than the illuminated. Fitting explained this with the assumption

> . . . that the polar opposition (polarity), which is induced by an external stimulus in all parts (cells) of the organ of perception, is spread out through the living tissues in a physiologically radially symmetrical zone of response. There is no lateral polarity; all the parts (including all the cells) which made up the path of stimulus conduction become "polarized" (longitudinally) in the same way. Because of this the responding zone gives rise to a curvature, either positive or negative, which is determined solely by the direction of this polarity. The polarity is dependent indirectly upon the external stimulus. Curvature increases until this "polarity" is removed again, according to the circumstances.

Stated in other words: The individual cells of the unilaterally illuminated tip become polarized so that a difference arises between the front and the back side, and this polarity is transmitted to the cells in the darkened basal portion.

Boysen Jensen.—In 1907, before Fitting's work was published, Boysen Jensen began experiments on the processes of stimulus conduction in the Avena coleoptile. It was found that the conduction of a stimulus from the unilaterally illuminated tip to the darkened basal portion could be arrested by an incision upon the back side, while this was not the case when an incision was made

on the front. These investigations were continued in Pfeffer's laboratory in Leipzig in 1909 and were repeated later with the same results in the plant physiology laboratory in Copenhagen. The method of investigation (Boysen Jensen, 1910, 1911) was briefly as follows: Avena seedlings were grown singly in small glass vials (10 by 2.5 cm.) filled with soil at a temperature of 20°C. in a darkroom. When a length of 2 to 3 cm. was attained, the coleoptiles were used; the experiments were performed in a darkroom with a humidity of 50 to 60 per cent. The light source was a Nernst lamp placed approximately 100 cm. from the experimental plants. Rectangular pieces of black paper about 9 cm. high were wrapped around the culture dishes to darken the basal portion of the plants (Fig. 3). Two screens held in place with a rubber band were placed around each glass; one screen extended to the bottom of the glass, while the other could be moved up and down and adjusted to the desired height. Even when these were not closed at the top, control experiments showed that light penetrating from above did not produce a phototropic curvature.

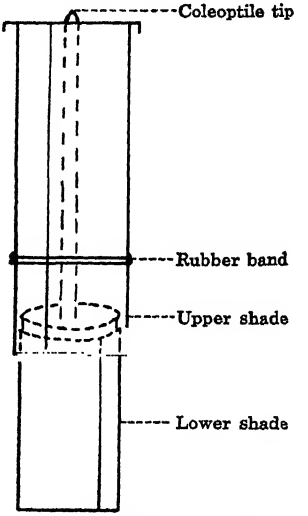


FIG. 3.—Method of darkening *Avena* seedlings. The culture dish is covered with cylindrical paper shades. The tip of the coleoptile protrudes through an opening in the top.

Transverse incisions were made with a sharp scalpel 2 to 3 mm. below the tip and extending about to the middle of the coleoptile. The basal portion of the coleoptile was darkened, and only the apical 1 to 2 mm. portion was illuminated unilaterally. These light exposures produced phototropic curvature in the darkened basal portion only when the incision was on the front side of the coleoptile and not when it was on the back (shaded) side. Next it was shown that the absence of phototropic curvatures in coleoptiles with an incision on the side away from the light was not due to the fact that the experimental plants had lost either their sensitivity to light or their ability to respond in the basal region. Coleoptiles wounded on the side toward the light showed marked

stimulus conduction and phototropic curvature in the basal portion, proving that they had not been affected by the incision. Coleoptiles with cuts on the back showed curvature in the basal region when the tips were darkened and the bases illuminated from the front.

Searching for an explanation of the disagreement between Fitting's investigations and those of Boysen Jensen, the latter repeated the experiments under the same conditions as those used by Fitting. The plants were kept in a saturated room, and their basal portions were darkened with the type of screen used by Fitting. Boysen Jensen then obtained the same result as Fitting, for the coleoptiles curved toward the light even when the incision was on the back side. Curvature did not occur, however, if a small piece of mica was inserted into the incision in the back side of the coleoptile (Fig. 1). If a thin transverse section from a Calamus stem was inserted in the cut instead of a piece of mica, then the stimulus was conducted past the incision.

These experiments may be summarized as follows: When a transverse incision is made on the same side of the coleoptile that is unilaterally illuminated, there is invariably a conduction of the stimulus from the unilaterally illuminated tip to the darkened basal portion. If, however, the incision is made upon the back side, stimulus conduction takes place only when the coleoptile is in a saturated atmosphere or after the wound surfaces become closely pressed together. Even in a saturated atmosphere, conduction is checked by the insertion of a thin piece of mica; it is not checked by insertion of a thin section of the living Calamus stem, which has large bundles and permits the passage of water and dissolved substances. These results are readily explainable on the assumption that the stimulus is conducted upon the shaded side of the coleoptile and that it can be transmitted across an incision.

Pfeffer was sceptical of the correctness of this theory, and so Boysen Jensen carried the investigations further. A deep incision was made upon the back side of the coleoptile so that only a very small connection remained between the tip and the basal portion on the front side. In this case, also, a transmission of the stimulus could be demonstrated, provided a close contact existed between the cut surfaces. Pfeffer maintained that so long as the tip is connected by any living substance with the basal region,

conduction of a stimulus over the incision is not proved beyond objection. There remained the problem of demonstrating that conduction of the stimulus can take place even when the living connection between the tip and base is completely destroyed.

Accordingly, two wedgelike incisions were made in the coleoptile about 1 cm. (or less) from the tip. Then the tip was removed, and the upper part of the foliage leaf was taken off to about 2 mm. above the wound. A small drop of gelatin solution was placed upon the cut surface of the coleoptile stump, and the tip was replaced in its original position, being held there by a ring of cocoa butter. When this replaced tip was unilaterally illuminated above the level of the cut, a decided positive phototropic curvature appeared in the darkened basal region (Fig. 1). This demonstrated beyond question that the stimulus could pass over an incision. Conduction of the phototropic stimulus was found to pass downward also when the cut surfaces were separated from each other by a thin section of Calamus. Similar experiments on the conduction of the stimulus in negatively geotropic curvature in the Avena coleoptile were carried out with the same result.

From these investigations it became clear that conduction of the stimulus in phototropic curvature takes place by the *downward movement of a growth-promoting substance* upon the back (shaded) side of the coleoptile.

Paál.—The correctness of Boysen Jensen's work was questioned by van der Wolk (1911). Later the experiments were repeated and confirmed by Boysen Jensen and many other investigators using somewhat different methods. Paál (1914, 1918) worked with coleoptiles separated from the seed and from the primary foliage leaf. After these empty coleoptiles had been placed in damp sand, the tips were cut off by smooth incisions and then replaced tightly. In about 88 per cent of the experiments a stimulus conduction from the unilaterally illuminated tip to the darkened basal region was demonstrated. Paál made the additional discovery that if the excised tip was placed on only one side of the coleoptile stump (Coix), greater growth occurred on the side beneath the replaced tip, and the coleoptile exhibited a marked curvature (Fig. 1). He replaced a decapitated tip of Avena in its normal position, inserting a piece of mica across one half of the stump so that tip and base were in contact only on one

side. A curvature developed toward the side with the mica plate. By inserting platinum foil between the tip and the base, transmission of the phototropic stimulus was inhibited. In this way, Paál proved that the stimulus was not electrical.

Stark and Drechsel.—An advance in technique was reported by Stark (1921*a*) who applied unilaterally to a coleoptile stump a block of agar which contained expressed sap from Avena coleoptiles (Fig. 1). Curvature followed. Stark and Drechsel (1922) perfected another method in which the coleoptiles were unilaterally cut a few millimeters from the tip with a sharp scalpel, the tip was then removed, and the exposed primary leaf was carefully pulled out, leaving the empty coleoptile attached to the seed. By this method they investigated transmission of the phototropic stimulus from a stimulated tip into the base of the same individual or different individuals of the same species or different species and genera. The desirability of a chemical analysis of the growth-promoting substance and a comparative study of photo- and geotropic reactions as a means of obtaining a unified explanation of the two responses was recognized. Stark's investigations will be discussed at some length later, as will those of Seubert (1925), who found that saliva, diastase, and malt extract were growth promoting.

Formulation of the Growth-substance Explanation.—After Boysen Jensen's results had been confirmed, there was some criticism of his experimental methods, and the suggestion was made that he had only hypothecated the existence of a growth-promoting substance in the Avena coleoptile, while Paál had demonstrated it. Boysen Jensen discusses this as follows.

> It is certainly not superfluous to see whether or not my experiments in the years 1909–1910 were without objection and what conclusions were drawn from these investigations.
>
> With regard to the first point, Paál maintained that the general set-up in my experiments with darkened coleoptiles was not reliable and that the experimental plants were insufficient in number.
>
> In considering the first point, it must be said that over one-half of my experiments were carried out with paper tubes as used by Rothert and Fitting. These are more reliable than the method used by Paál, and the usefulness of this method has been proved by controlled experiments both by Fitting and by myself. In addition, I could demonstrate a conduction of geotropic stimulus from the separated tip to the basal

region even in plants that had not been subjected to light. This objection can then surely be overruled.

The next question is whether a sufficient number of experiments were carried out. I disregard here all incision experiments and confine myself to the experiments with removed and replaced tips. Thirty-five plants were used for the experiments on phototropic stimulus conduction; of these 25 reacted positively, 9 remained straight, and 1 showed

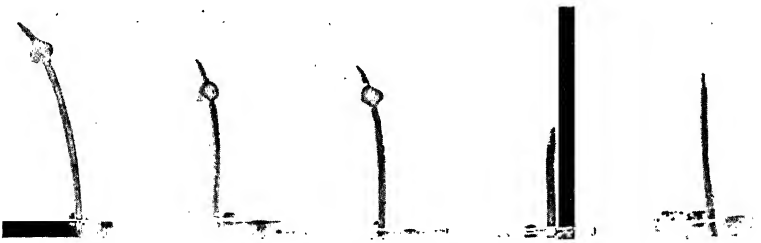


FIG. 4.—Phototropic curvature in decapitated *Avena* coleoptiles. The tips were replaced upon the three plants at the left, while the two plants at the right served as controls. The plants with tips curved toward light.

a weak negative curvature. Furthermore, a geotropic stimulus conduction was demonstrated in 12 plants (number of experimental plants, 12). Information as to the strength of the curvatures is given by photographs in my papers of 1911 and 1913 (Figs. 4 and 5). The curvatures are so strong and the percentage of curved plants so high that the published material is sufficient to prove a stimulus conduction from the removed tip to the basal portion.

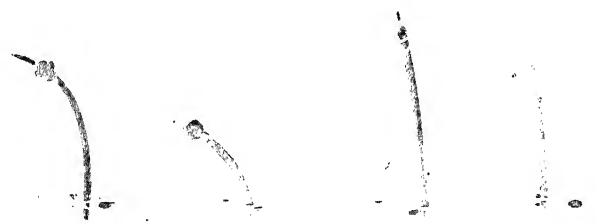


FIG. 5.—Geotropic curvature in decapitated *Avena* coleoptiles. The tips were replaced upon the two plants at the left, while the two plants at the right served as controls. The plants with tips curved away from the force of gravity.

Relative to the conclusions that I drew from the experiments published in 1911, I should like to cite the following from that paper:

"We are able, therefore, to represent the facts more or less as follows. Under the influence of the action of unilateral light there is produced a differentiation between the front and the back faces at the tip of the coleoptile (and not, as Fitting thought, in the individual cells of the

coleoptile). We shall put aside provisionally the question of whether this differentiation is of a 'physical' or 'chemical' nature. The stimulus is transmitted from the back side of the tip down the length of the back side of the coleoptile. Since, as I have shown, there is no modification in the rate of growth on the front side of the coleoptile, the positive phototropic curvature must result from the accelerated growth rate which is induced by the light stimulus."

In his discussion of the nature of the transmission, Boysen Jensen (1911) wrote further:

"It seems to me that my studies of the transmission of the stimulus in the Avena coleoptile render it probable that in this case the transmission of the stimulus is of a material nature and produced by contraction changes in the tip of the coleoptile. In every case it would seem necessary to resign oneself to a hypothesis according to which the transmission of the stimulus in Avena would be due to physical causes (changes of pressure, etc.), which is perhaps the case for Mimosa; in fact we have seen that the stimulus can be transmitted across an incision made in the coleoptile. For other reasons it is thought that the transmission of the stimulus is of a chemical nature. As may be recalled, the condition for transmission across an incision was that the edges of the wound were kept humid and held one against the other in a way to favor as much as possible transmission of a substance or of ions across the incision. Another reason: It has never been proved that the transmission of the stimulus could take place under water. The water under such a condition should prevent this transmission, which can be explained only in a hypothesis where the transmission of the stimulus would have been due to the migration of a substance or of ions, which would diffuse into the water and no longer act."

It may be said, therefore, that in 1911, Boysen Jensen's conception of phototropic curvature in the Avena coleoptile was the following: Under the influence of unilateral light, a polarity is formed in the coleoptile tip which is associated with an unequal distribution of a substance upon the front and back side of the coleoptile. The substance in question migrates down the back side (he used the expression "migration," since it seemed clear, even at that time, that it could not be a process of diffusion) and causes an acceleration of growth upon the back side in the basal region, which produces a phototropic curvature. This conclusion seemed the only possible one. The existence of a growth substance in the Avena coleoptile during photo-

tropic curvature was demonstrated, therefore, through these investigations.

Contemporaneously with these studies, in the years 1909 and 1910, Fitting published two works in which it was shown that orchid pollinia contain a substance that produces a swelling of the gynostemium. According to Fitting (1910), this substance is a hormone; and according to the more recent investigations of Laibach (1932) and of Laibach and Maschmann (1933), it is probably identical with the growth substance of the Avena coleoptile.

In the past few years, marked advances have been made in our knowledge of the occurrence, movement, and quantitative determination of the plant-growth substances (Went, 1928*a*). Recent outstanding contributions to the chemistry of the subject (Kögl, Haagen Smit, and Erxleben, 1932–1935) have opened up new phases of the general investigation which may become valuable in horticultural practice (Bouillenne and Went, 1933; Hitchcock and Zimmerman, 1935; Cooper, 1935). Detailed discussion of the more significant aspects of the growth-substance problem will be presented in the chapters that follow.

SUMMARY

The starting point for growth-substance investigations was the demonstration of a growth-promoting material in the tip of the Avena coleoptile, as shown by phototropic curvature. The brief historical sketch which has been presented here indicates that the growth-substance explanation of photo- and geotropism had its origin many years ago. About one-quarter of a century has elapsed since a hypothesis was suggested according to which a stimulus substance in the coleoptile was displaced by the effect of unilateral light, or gravity (Boysen Jensen). Other contributions to the solution of the problem followed (Paál, Stark, and Seubert). A new impetus was given to the subject when Went (1927, 1928*a*) published his method of procedure for extracting growth substance and demonstrating the quantitative relationship between it and growth in the Avena coleoptile. An ever increasing fund of knowledge about hormone activity is continually extending our understanding of tropisms and the whole problem of normal growth.

CHAPTER II

DETECTION AND QUANTITATIVE DETERMINATION OF GROWTH SUBSTANCES

In demonstrating the presence of growth substances, the coleoptile of the Avena seedling has been used almost exclusively as a test object. Its structure and sensitiveness to stimuli make it suitable for quantitative tests as well as qualitative demonstrations. A minute amount of a growth hormone applied unilaterally near the tip of a coleoptile brings about increased growth on the side receiving the growth substance, and this produces a growth curvature. The amount of curvature can be used, within certain limits, to indicate the concentration of the applied growth substance. In a similar way Cephalaria seedlings have been used as quantitative test objects (Söding, 1935*a*, *b*). Other methods and numerous other plant organs are equally useful for qualitative demonstrations (see Figs. 17, 37, 39).

THE TEST FOR THE PRESENCE OF GROWTH SUBSTANCE WITH THE AVENA COLEOPTILE

The Culture of the Avena Seedling for Use as a Phytohormone Test Object.—A genetically uniform variety of *Avena sativa* has been used almost universally in the plant-hormone work of the past few years. It is obtainable from Dr. E. A. Åkerman of Svalöf, Sweden, and is known as *siegeshafer*, or victory oats. While other uniform strains may be used just as successfully, there is an important advantage in all workers having genetically comparable test material. The variety *gul naesgaard* is used in the Copenhagen laboratory.

Culture Conditions.—The generally accepted culture method necessitates a darkroom for growing the Avena seedlings and for carrying out the quantitative determinations, although Söding (1935*a*) has recently described a daylight method. The arrangement of such a darkroom has been described repeatedly in the literature (*e.g.*, Linsbauer, 1922; Went, 1928*a*; Nuernbergk,

1932*b*). It is best to have controlled temperature and humidity; fluctuations of 0.5°C. and ±1 per cent relative humidity make little difference in most experiments. The laboratories that have such controlled conditions usually maintain the temperature at 25°C. and the relative humidity at 90 per cent. If such a laboratory is not available, a thermoregulator will provide temperature control for a darkroom, and a suitable humidity may be obtained by placing the experimental plants under bell jars. Light for the darkroom must be *phototropically inactive*, which means that wave lengths longer than 5,500Å. may be used. Ruby glass or filters such as Corning 246 or Schott OG-2 are satisfactory.

The culture of the seedlings involves certain difficulties, perhaps the greatest of them being that at times the first internode ("mesocotyl" of the older literature—see Avery, 1930) elongates under the coleoptile and by its nutations makes a whole series of plants useless. Numerous factors have been suggested as the cause of this elongation, among them low temperatures (Blaauw, 1909), low moisture content of the soil (Noack, 1914), strong carbon dioxide content of the atmosphere (Maria de Vries, 1917), etc. Through the investigations of Lange (1927, 1929), Beyer (1927*b*), duBuy and Nuernbergk (1929*b*), and Hamada (1929, 1931) it has been shown that elongation of this internode in Avena can be suppressed by illuminating the seeds during the soaking period (see below) with bright daylight. Rothert (1894, p. 27) had already pointed out that temporary illumination was effective in checking the development of this internode. DuBuy and Nuernbergk (1929*a*) showed that its elongation can be checked also by heat radiation.

It has been shown that nutations may occur in the coleoptile (Bremekamp, 1925; Lange, 1925; Pisek, 1926; Beyer, 1927*b*; Lange, 1933), but these have no role in the usual culture difficulties. They become apparent only when the plants are put on the clinostat. These curvatures take place in the plane of symmetry of the plant, either away from the seed (*e.g.*, victory oats) or toward the seed (*e.g.*, orien oats). In addition, occasional torsions may appear in the coleoptile (Beyer, 1927*b*; Tammes, 1931); these have no special significance in the culture of experimental plants for hormone-test objects, nor have the photonastic curvatures described by Lange (1933).

Culture Methods.—Whether the Avena seedlings are grown in soil, sawdust, or water culture, the preliminary treatment is about the same. The glumes may or may not be removed for soil or sawdust culture; the seeds are soaked in water for 2 to 4 hours, after which they are placed in petri dishes on moist filter paper and allowed to remain for about 36 hours. (If illuminated for a few hours at the beginning the first internode remains short.)

For Boysen Jensen's soil-culture method, the seeds are removed from the petri dish and placed in glass vials, 20 by 100 mm., filled with screened, sandy garden soil (Fig. 6). The soil should be well-watered so that no further watering will be necessary but not

FIG. 6.—Curvatures produced by placing *Avena* coleoptile tips unilaterally upon decapitated coleoptiles.

too wet, for the plants then become less sensitive. Each vial contains but one seed, and 25 to 30 vials are held together by an elastic band. These are moved into the darkroom and placed in a petri dish, then covered with a small bell jar so that the air will remain saturated. Under the usual conditions in the darkroom at a temperature of 21 to 21.5°C., the coleoptile appears in two days. Then the bell jar is removed, and the coleoptiles continue their growth under low-humidity conditions for 24 hours. In this time they attain a length of 15 to 25 mm. and are ready for use.

Navez and Robinson (1932*b*) planted the seeds with glumes removed in sterile, purified, maple sawdust contained in glass vials 15 by 25 mm. The sawdust retains about 4.3 times its weight of water. The seed is planted dry, embryo side upward, and inclined about 10 deg. from the vertical. The end of the seed away from the embryo may or may not be allowed to protrude slightly above the level of the sawdust. In this method, germination is allowed to proceed in a light-tight chamber at a tempera-

ture of 22 to 22.5°C., and after 72 hours the seedlings are ready for use; they are then 25 mm. long on the average.

Went (1928*a*) used plants grown in water culture. After the preliminary treatment mentioned above, they are transferred to the darkroom and allowed to remain in the germinating dishes until the seedling roots are a few millimeters long. They are placed then in glass holders over zinc or glass trays of water, as indicated in Fig. 7. At a temperature of 25°C. and relative

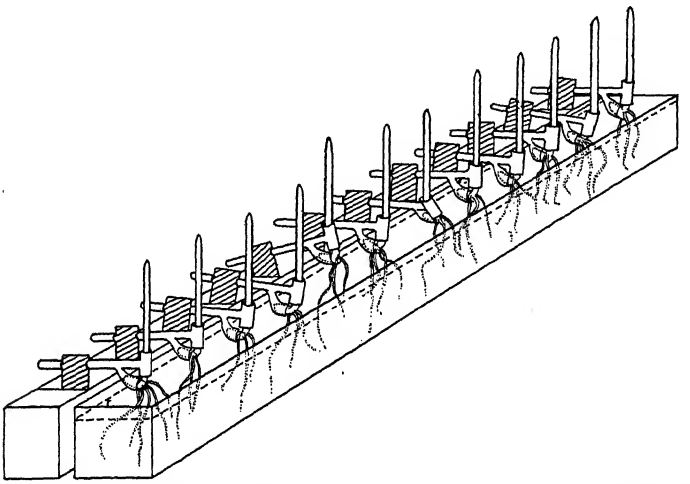


FIG. 7.—Diagram illustrating the water culture method of growing *Avena* seedlings as test objects for making growth-hormone determinations. The oat seedlings are supported in glass holders held in brass clamps; these fit firmly into slots in a wooden block. The roots of the seedlings dip into a tray of water. Orientation of the coleoptiles may be accomplished by adjusting the positions of the brass clamps and glass holders. (*Modified from Went*, 1928*a*.)

humidity of 90 per cent, the coleoptiles are ready for use after about 30 hours.

Each of the methods described has its advantages and disadvantages, which must be evaluated at the time when a particular experiment is contemplated. Culture of the seedlings in vials may facilitate working with individual plants but increases the number of manipulations when many tests are being made. Difficulty is encountered in properly orienting the coleoptiles for uniform application of the plant parts or agar blocks to be tested. The fixed position of the seedlings in the soil or sawdust, which raises this difficulty, turns out to be an advantage if much

handling of the containers is necessary. Fine soil or sand may pack tightly enough to hinder proper aeration of the roots and thus inhibit growth of the seedlings. Sawdust, however, which provides excellent aeration and adequate room for root growth, must be boiled sufficiently to free it of toxic substances, such as resins and tannins.

In using liquid culture methods, several factors must be considered. Roots immersed in solution may not be sufficiently aerated for vigorous growth. Attention should be given to the solution bathing the roots—whether it shall be distilled water or some nutrient mixture. In water culture it is necessary to handle the seedlings twice, once when they are placed in dishes to germinate and again when they are mounted in holders. This, however, provides an opportunity for selecting the uniform plants for use in testing, and those which are not satisfactory may be discarded. The advantages, which may outweigh the difficulties of the method are that the seedling holders allow for easy handling of many test plants, for perfect orientation of each seedling so that its coleoptile is vertical and for convenient photographing of the resulting curvatures. For any quantitative work these qualifications are of distinct advantage. For most qualitative studies, the less complicated methods of soil or sand culture are entirely adequate.

Preparation of the Avena Coleoptile for Use.—The small quantities of growth substances in plant organs make difficult direct proof of their presence by chemical means. For the purposes of many biological experiments it is satisfactory to obtain indirect evidence of their existence by their activity in certain measurable growth reactions. Growth substances, in common with other hormones and activators, produce in the living organism responses out of all proportion to the size of the stimulus. Although they have been extracted and purified from many plant sources, the usual method of detecting them is by means of biological indicators. For this purpose the Avena coleoptile has been used more extensively than any other organism. Its culture up to the time of the test has been described, and now some of the methods of procedure will be outlined.

Decapitation and Unilateral Application.—Coleoptiles to be used for test purposes should have attained a length of 25 to 40 mm. before they are decapitated in the following way (Fig. 8*B*):

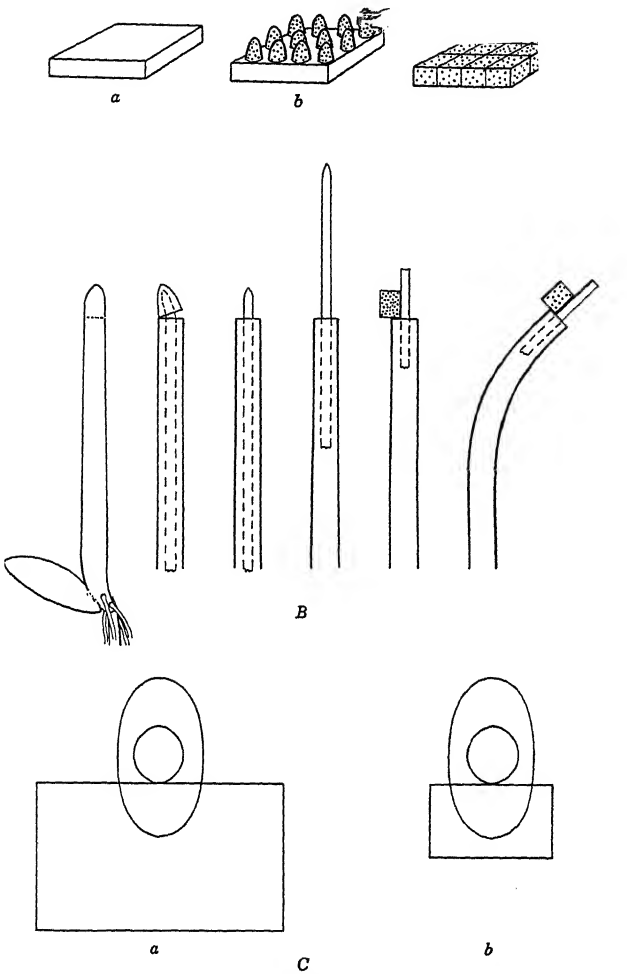


FIG. 8.—Technique of testing for growth hormone with agar blocks applied to the decapitated coleoptiles of *Avena* seedlings. *A*, diffusion of growth hormone into agar; *a*, agar plate; *b*, plant material (e.g., coleoptile tips) to be tested for the presence of growth hormone is placed in contact with agar for 2 hours; *c*, the agar plate containing growth hormone is cut into small blocks. (*After Went*, 1928*a*.) *B*, decapitation of *Avena* coleoptile and unilateral application of agar block. The tip of the coleoptile is removed and the foliage leaf is pulled out part way and cut off; a small portion is left extending from the apex of the coleoptile stump. An agar block containing the hormone to be tested is placed over a vascular bundle on one side of the cut apex (see Fig. 13). The ensuing curvature is proportional, within limits, to the concentration of growth hormone in the agar block. *C*, end view of decapitated coleoptiles in contact with agar blocks. The contact area is the same in both *a* and *b* though the volume of one block is 8 times that of the other. (*After Thimann and Bonner*, 1932.)

a unilateral incision is made (Stark and Drechsel, 1922) 2 to 3 mm. from the tip with a sharp scalpel or razor blade; the tip then is removed by a slight jerk with forceps or the thumb and forefinger. Went (1928*a*) removed 5 to 8 mm. of the tip when decapitating. Various sorts of instruments have been made (Went, 1928*a*; van der Weij, 1931; duBuy, 1933) to aid in decapitation. The primary leaf protrudes from the cut surface of the coleoptile stump after decapitation. It may be pulled loose and carefully drawn out with a pair of forceps until only the basal 5 mm. of it remain inside the coleoptile; the protruding portion then is severed about 5 mm. above the tip of the coleoptile stump.

When the coleoptile has been prepared as above, the object to be tested, that is, a small plant organ, portion of an organ, or agar block, may be applied unilaterally as in Fig. 1 (Paál and Stark) and Fig. 8*B*. If the object contains substances that influence growth, they migrate down one side of the coleoptile and cause a curvature.

The actual procedure, from the time of decapitation on, varies with different workers: Immediately after decapitation (of 15 to 25 mm. coleoptiles), Boysen Jensen applies unilaterally the object that is being tested for the presence of growth substance. He cautions that while curvature is taking place the humidity must not be so high that the plants guttate and disturb the object being tested or so low that the object dries up; plants grown in soil are best placed under bell jars which are partly lined on the inside with moist paper. The rapidity with which curvature takes place depends upon the temperature; in Boysen Jensen's laboratory (Copenhagen) the work is carried out at 21.5°C. At this temperature the maximum curvature occurs after 2½ to 3 hours. The use of a longer experimental period is not recommended, since "physiological regeneration" of the tip can influence the reaction.

In the Utrecht laboratory the coleoptiles are decapitated when 40 to 60 mm. long (Went, 1928*a*) and allowed to stand 40 minutes; at the end of this time, all coleoptiles that are not perfectly straight are eliminated. Any guttation fluid that may appear at the tip of the decapitated coleoptile is removed by "blotting" with a small piece of filter paper. The object to be tested is unilaterally applied and allowed to remain for 120 minutes, at

the end of which time the degree of curvature may be measured, as described later. The temperature of the laboratory at Utrecht is maintained at 22°C. and at a relative humidity of 90 to 95 per cent. If it is desirable to work with greater numbers of test plants, the coleoptiles may be decapitated a second time 60 or 90 minutes after the first decapitation (van der Weij, 1931). The time schedule mentioned is important only for the quantitative work discussed later.

Growth Curvature of the Coleoptile.—The appearance of a curvature after the application of the unknown material is evidence for the presence of growth substance. A negative curvature (bending *away* from the side with the applied object) indicates a *growth-promoting* substance, whereas a positive curvature shows that *growth-retarding* substances are present (Fig. 1) (Stark). Quantitative methods for determining the amount of growth-promoting substances present are described in the last part of this chapter. If no growth curvature occurs, it means either that the object being tested contains no growth substance or that there is some factor which disturbs either the transfer of the growth substance or its effectiveness after entrance into the Avena coleoptile. Mention of cases in which the latter is true is made at the end of the chapter.

Preparation of the Material to Be Tested. *Direct Application of Plant Parts to the Decapitated Avena Coleoptile.*—Many plant organs or parts of organs such as coleoptile tips, coleoptile cylinder segments, root tips, etc., may be tested for the presence of a growth substance by placing them (Figs. 6 and 8*B*) unilaterally upon decapitated Avena coleoptiles (see Paál, 1918; Stark, 1921*b*; Nielsen, 1924). This is the simplest method and the first one employed in attempting to detect growth substances in some new object.

Application of Material to Be Tested in Agar and in Lanolin.—Frequently it is not feasible or desirable to apply the plant parts to be tested directly to the Avena coleoptile. Other methods depend upon the fact that growth substances are soluble in water, alcohol, and ether, and that they are stable in agar and lanolin (wool-fat) paste. With some of these methods it is possible to concentrate the extract obtained from a quantity of plant material before making the biological test and thus demonstrate that growth substance is present even though only in small amounts.

1. DETECTION OF GROWTH SUBSTANCE IN FLUIDS.—If a fluid is to be tested for growth substance, the reaction must be weakly acid; one neutralizes where necessary with sodium bicarbonate and adds a little citric or acetic acid (0.2 cc. per liter). If the solution is very pure, it may be necessary to add some potassium chloride (169 mg. per liter) (Kögl and Haagen Smit, 1931, Mitt. I). The substratum is prepared in the following way: A computed amount of agar is carefully washed with tap water for 24 hours; afterward the agar plus the absorbed fluid is weighed again, and enough water is added to produce a 3 per cent agar. The solution

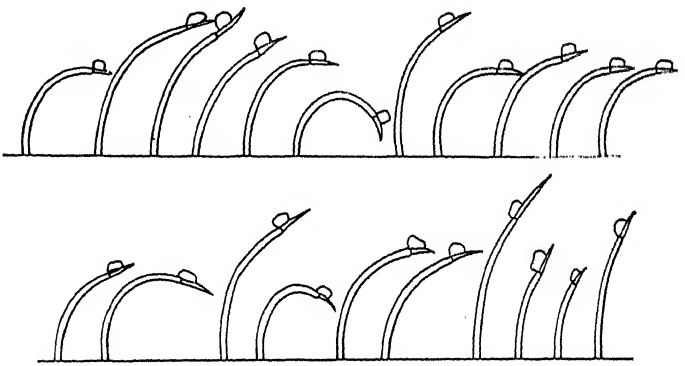


FIG. 9.—Curvatures resulting from application of agar blocks containing saliva to one side of decapitated coleoptiles. (*After Seubert*, 1925.)

which is being investigated for growth substance is then mixed with an equal amount of the substratum. After solidification, small blocks of equal size are cut out and placed unilaterally upon decapitated Avena coleoptiles (for size of blocks, see description under quantitative determination). Instead of mixing the solution to be tested with melted agar, agar blocks can be placed in the solution for 1½ hours. Through control experiments it can be shown that the agar substratum has no effect upon the Avena coleoptile. This method was originally used by Stark (1921*b*) and later by Nielsen (1924), Seubert (1925), and others (Fig. 9).

It is possible also to detect the presence of growth substance in a fluid by mixing it in various proportions with lanolin (Laibach, 1933*b*). The lanolin growth-substance paste may be applied unilaterally to intact coleoptiles; if bending occurs, it may be concluded that growth substance is present (Fig. 37*A*).

2. DETECTION OF GROWTH SUBSTANCE IN POLLEN.—A mixture of pollen and 1 cc. water, weakly acidified with acetic acid, may be applied in a small chamber around the stump of a decapitated coleoptile. Growth may be measured interferometrically (Fig. 28) (Laibach and Kornmann, 1933*a*) or in any other suitable manner. Pollen mixed with agar (weakly acidified) may be cut into blocks and applied unilaterally to intact coleoptiles (Laibach and Kornmann, 1933*a*). Pollen may be suspended in lanolin by mixing thoroughly in the proportion of 50 mg. air-dried pollen, 1 cc. water (weakly acidified), and 1 g. anhydrous lanolin. The mixture may be applied to various kinds of test objects, where it will induce bending (Fig. 37): intact or decapitated coleoptiles, epicotyls of *Phaseolus multiflorus*, aerial roots of various species, petioles of Coleus, etc. This method of preparation and application is very useful because the growth substance is given off into the plant very slowly, and the lanolin does not dry out (Laibach, 1933*b*).

3. DIFFUSION OF GROWTH SUBSTANCE INTO AGAR.—Went (1928*a*) demonstrated that growth substance would diffuse out of decapitated coleoptile tips if the latter were allowed to remain standing on 3 per cent agar blocks for approximately 2 hours (Fig. 8*A*). Since this method was first described, growth substances have been "diffused" out of numerous other plant parts. The agar blocks are then applied unilaterally to decapitated coleoptiles as described above. Details of Went's procedure are to be found under the discussion of quantitative determination.

4. DIFFUSION OF GROWTH SUBSTANCE INTO DEXTROSE AGAR.—Boysen Jensen (1933*b*) found that it was impossible to obtain growth substance from roots by standing the decapitated root tips on 3 per cent agar, but satisfactory demonstrations were made possible by the use of a dextrose salt agar of the following composition: 3 g. agar, 10 g. dextrose, 0.1 g. calcium nitrate, 0.025 g. potassium monohydrogen phosphate, 0.025 g. magnesium sulphate, a trace of ferric chloride, and 100 cc. water. The agar blocks must be made up fresh the day that they are to be used, and this is done most easily if 10 cc. of the agar mixture is spread out upon a warm glass plate (size 10 by 10 cm.). Blocks can be cut from this by using parallel knives; the size of the blocks used is 2 by 2 by 1 mm. Upon such blocks root tips of *Zea mays* or *Vicia Faba* are placed for 2 to 4 hours; the blocks are

then moistened with a solution of 1 g. citric acid, 50 cc. alcohol, and 50 cc. water. If necessary, they may be kept for a time in the refrigerator and later applied unilaterally to decapitated Avena coleoptiles (Fig. 10).

5. DIFFUSION OF GROWTH SUBSTANCE INTO WATER.—Another method of extraction has been described by Gorter (1932). The pieces of plant, for example, coleoptile tips, are placed upon a layer of sand which is soaked with water. After a time they are removed, the water filtered off, and the sand washed repeatedly. The filtrate and rinsing water are either evaporated in a vacuum or extracted with ether. The residue from the evaporated ether

FIG. 10.—Curvatures of decapitated *Avena* coleoptiles resulting from application of agar blocks upon which root tips of *Vicia Faba* had been standing for 4 hours. (*From Boysen Jensen*, 1933.)

extract is dissolved in water which contains 160 mg. potassium chloride and 0.2 cc. glacial acetic acid per liter; agar blocks are placed in the solution, and later these are tested for growth substance in the usual way.

6. EXTRACTION OF GROWTH SUBSTANCE WITH ALCOHOL.—Growth substance can be extracted also from plant parts with alcohol. The alcohol which is poured off is concentrated in a vacuum, and the residue dissolved in an optional amount of water; this solution is investigated either directly after mixing with agar or after it has been purified with ether (the latter is described later).

7. EXTRACTION OF GROWTH SUBSTANCE WITH CHLOROFORM.—Thimann (1934) has described a method of chloroform extraction of growth substance from tissues. It consists, in brief, of killing the fresh material by immersing it in a small amount of chloroform, adding 0.1 *N* hydrochloric acid to the extent of about one-fifth the volume of the chloroform, and grinding the mixture thoroughly. The extract containing the growth substance is

poured from the residue into a small separatory funnel where the aqueous layer is drawn off and placed again with the ground tissue. A small amount of chloroform is added, and the mixture is ground again; the extract is placed in the separatory funnel as before, and the aqueous layer is again returned to the ground tissue. The same procedure is repeated a third time. The total chloroform-water mixture then is shaken thoroughly, and the chloroform layer separated off. The latter contains the growth hormone and is transferred to a small test tube and evaporated off. The minute amount of lipoidal material which remains is taken up in a very small volume of water, to which an equal volume of 3 per cent agar is added. If 0.15 cc. each of water and agar are used, the resulting 0.3 cc. may be cast into a small block 8 by 10.7 by 1.5 mm. (there is always some loss in volume, and the amount left will approximately fill a mold of this size), which in turn may be cut into 12 smaller blocks of equal size if quantitative determinations are desired (see Went's quantitative method).

8. EXTRACTION OF GROWTH SUBSTANCE WITH WATER.—Thimann also tried water extractions with fair success but found that the growth substance was rapidly inactivated by oxidizing enzymes.

QUANTITATIVE DETERMINATION OF GROWTH SUBSTANCES

Determination of Growth Curvature Produced by the Unilateral Action of a Growth Substance.—After demonstrating that growth substance would "diffuse out" of living parts of plants into agar, Went (1928*a*) showed that if agar blocks were placed unilaterally on decapitated coleoptiles (after the method of Stark, 1921*b*), there would result a curvature proportional within certain limits to the amount (later shown to be concentration) of growth substance in the block (Fig. 2) (Went). The investigations of Nielsen (1930*a*, *b*) and Dolk and Thimann (1932) have shown a similar direct relationship between concentration of growth substance and coleoptile curvature (Fig. 11), and it is upon this simple fact that quantitative determinations depend.

The tests should be carried out in a darkroom under phototropically inactive light.

Boysen Jensen's Quantitative Method.—This involves a determination of the difference in length of the convex and concave

sides of a curved coleoptile. The concentration of growth substance necessary to bring about a certain difference in the length of the two sides is designated as one WAE (*Wuchsstoff Avena*

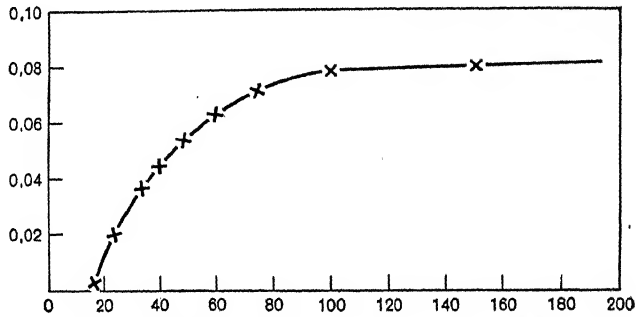


FIG. 11.—Curve showing relationship between amount of bending in the *Avena* coleoptile and the concentration of "rhizopin" applied unilaterally in agar blocks. Ordinate: *d* value. Abscissa: relative concentration of the growth substance. (*After Nielsen*, 1930.)

Einheit—see 5, page 29). The method may be outlined briefly as follows:

1. The test plants are grown in soil cultures and decapitated as outlined under "culture methods" (p. 18).

2. The agar blocks 2 by 2 by 1 mm. containing the growth substance (see 4, p. 25) are applied unilaterally to decapitated coleoptiles and are allowed to remain for 3 hours.

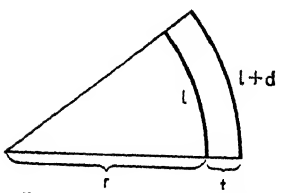


FIG. 12.—Diagram showing derivation of *d* value. *l*, length of coleoptile, inner curved side; *d*, difference in length between inner and outer curved side; $l + d$, length of outer curved side; *t*, diameter of the coleoptile; *r*, radius of curvature.

3. The curvature produced by the unilateral effect of growth substance is the result of a difference in length between the convex and concave sides; this difference, *d*, is the result of unequal rates of growth. When measured in millimeters, this difference becomes a clear expression for the effect of growth substance upon the coleoptile (if the approximate growth rate of decapitated coleoptiles is known). The *d* value may be determined by the method employed by Purdy (1921) (see Fig. 12). If *l* is the length of the curved part of the *Avena* coleoptile, *d* the difference in length, *t* the diameter of the coleoptile, and *r* the radius of the curvature, the following equations are obtained:

$$\frac{r}{r+t} = \frac{l}{l+d}; \qquad \frac{r}{t} = \frac{l}{d}; \qquad d = \frac{tl}{r}.$$

l is determined with millimetric paper, and t with a micrometer screw; the diameter of the organ is usually about 1.5 to 1.7 mm. To measure r, arcs with various radii (0.6 to 10 cm.) are drawn upon paper. If the curved Avena coleoptile is compared with the arcs, the radius of curvature of the coleoptile can be measured, and the d value computed in millimeters from the equation given (p. 28). (For applications of the radius of curvature for curvature measurements, see Rothert, 1894.)

4. The number of experimental plants to be used for a determination depends, of course, upon the degree of accuracy that one wishes to achieve. Various experimental series of Nielsen (1930*b*) give detailed information concerning the fluctuations in magnitude of curvature in the experimental plants. From these data it has been computed that when d is equal to 0.56 mm., the standard deviation of a single measurement is about ± 0.09 mm. A mean error of about ± 0.03 mm. has been found when using 9 plants; the error is reduced to approximately ± 0.013 mm. with 50 plants. Measurements for general purposes of orientation can be made with about 6 to 8 plants; 10 to 12 and preferably 30 to 40 plants should be used for more exact measurements.

5. The unit of growth substance used in the Copenhagen laboratory (Boysen Jensen, 1931*b*) is that amount, dissolved in 50 cc. water plus 50 cc. 3 per cent agar, which will produce a d value of 1 mm. when the curvature of the Avena coleoptile takes place at a temperature of 21 to 22°C., and the magnitude of this curvature is measured after 3 hours. This amount is designated as a growth-substance Avena unit (*Wuchsstoff* A-*Einheit* = WAE). The size of the blocks should be uniform (2 by 2 by 1 mm.), although small deviations have no influence upon the size of the curvature (as Nielsen (1930*b*) and van der Weij (1932) have shown) since this is dependent upon the growth-substance concentration and not upon the amount of growth substance (see also van der Weij (1932) and Thimann and Bonner (1932) on concentration *vs.* amount); however, the amount of contact surface between the agar and the coleoptile should always be the same (Fig. 8*C*). The degree of curvature is much greater when the block is placed over a bundle than when it is placed on parenchymatous tissue (Laibach and Kornmann, 1933*b*); hence the block should be placed in contact with a vascular bundle if consistent results are to be obtained (Fig. 13).

6. If the growth-substance content of a solution is to be measured in WAE, a number of dilutions are made from the solution, *e.g.*, ½ (*i.e.*, 1 cc. solution + 1 cc. agar), ¼ (1 cc. solution, 1 cc. water, 2 cc. agar), etc., in order to find the dilution that produces a d value of about 0.5 mm. If, for example, the d value is 0.55 mm. with a ⅛ dilution, then the original solution contains 4.4 WAE in 100 cc.

7. If it is desirable to determine how much growth substance moves from a plant organ into an agar block in a definite time, the block must have a very definite size, such as mentioned above. The following will serve as an example for computation of the amount of extracted growth substance: If a root tip is placed upon a block of dextrose agar 4 $mm.^3$ in size and allowed to remain for 2 hours, and the block produces in the Avena coleoptile a curvature with a d value of about 1 mm., then the root tip has given off

1/50,000 WAE per hour. However, in practice if the *d* value is much greater than 0.5 mm., the direct relationship between *d* and growth-substance concentration (characterized by *d* values of 0.1 mm. or less up to 0.5 mm.) no longer holds. As a unit of the amount of growth substance given off into an agar block, the "tip hour" has also been proposed. The amount that comes from an Avena tip in one hour produces a curvature of about 15 deg. and

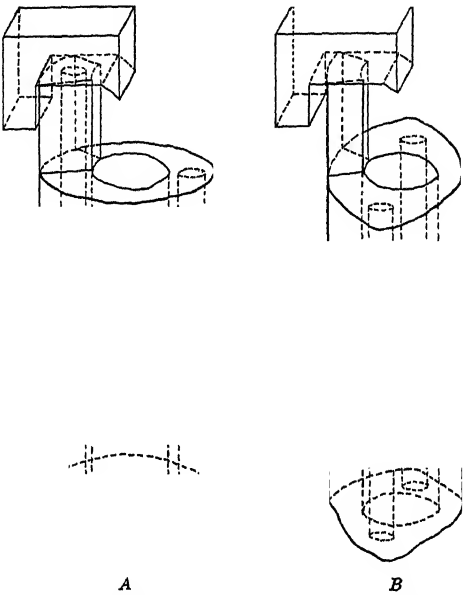


FIG. 13.—Unilateral application of agar blocks containing growth hormone to the cut surface of *Avena* coleoptiles, either in contact with a vascular bundle, *A*, or parenchyma, *B*. (*After Laibach and Kornmann*, 1933*b*.)

corresponds therefore to about 1.5 AE (see duBuy and Nuernbergk, 1932). (For a definition of AE see p. 33.)

Went's Quantitative Method.—This method involves a determination of the angle of curvature of the decapitated coleoptile on which has been placed the object to be tested. The measurement is made by means of a protractor, equipped as shown in Fig. 14. The concentration of growth substance necessary to bring about a curvature of one or more degrees is designated in various ways, as discussed on page 32. The method has been modified slightly by several workers since 1928, and the current procedure, assuming that the test plants have reached the proper size, is as follows:

1. Three per cent agar plates are prepared from agar which has been tested previously and found to be free from growth substance. DuBuy (1931) has shown that curvatures are reduced when higher concentrations of agar are used. Two sizes of agar plates are in common use: 8 by 10.7 by 1.5 (Dolk, 1930) and 8 by 6 by 1.0 mm. (Went, 1935*a*).

2. The coleoptile tips, portions of leaves, buds, or other plant parts to be tested for growth substance are freshly severed from the plant and allowed to stand proximal end downward on the rectangular agar plates for a period of 2 hours (Fig. 8*A*). They should be covered with a bell jar lined with moist paper throughout the period of diffusion.

3. After diffusion the rectangular agar plates are cut up into 12 equal blocks by means of a special cutting device: Dolk proposes that the plates

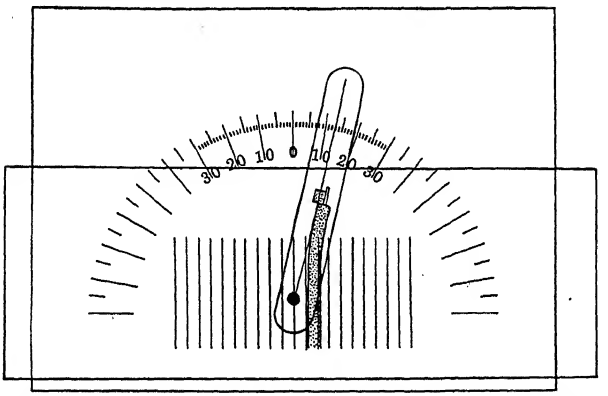


FIG. 14.—Method for determining the degree of curvature in an *Avena* coleoptile. The transparent celluloid protractor is placed over a shadow picture (Fig. 15) of the curved coleoptile and the angle of curvature is measured directly by matching the thin line on the celluloid arm with the axis of the curved organ. (*Modified after Went*, 1928*a*.)

8 by 10.7 by 1.5 mm. be cut into 12 blocks, each 2.67 by 2.67 by 1.5 mm. (10.7 mm.^{3}); Went suggests that the plates 8 by 6.0 by 1.0 mm. be cut into 12 blocks, each 2 by 2 by 1 mm. (4.0 mm.^{3}). Kögl and his associates cut the agar into blocks 2 by 2 by 0.5 mm. or 2 mm.^{3} Since it has been shown that size of block is not of great importance as long as the amount of contact surface is the same, any of the foregoing sizes is satisfactory. The larger blocks used by Dolk do not dry out so readily.

4. These 12 blocks are applied unilaterally to 12 of the previously decapitated coleoptiles (40 minute interval between decapitation and application of blocks). The time allowed for curvature to take place is 110 (Dolk and Thimann, 1932) to 120 minutes (Went, 1928*a*). The same procedure is followed with agar blocks from Thimann's chloroform method (p. 26) or with agar blocks made up with different dilutions of growth substance (p. 24). In determining the growth-substance concentration of a solution it is possible also to immerse the blocks in the unknown solution for 1 to 1½ hours and then proceed in the usual manner.

5. The rack of 12 test plants with their curved coleoptiles is placed directly in front of a sheet of silver-bromide paper, and a shadow picture is taken of the 12 coleoptiles (Fig. 15).

6. After developing the print, the curvatures are determined by measuringthe deflection of the coleoptile tips in degrees, a method first introduced by Simon (1912); Went (1928*a*) suggests a simple measuring protractor (Fig. 14) for this purpose, and the photograph provides a permanent record which can be referred to later if desired. Söding (1934) has described a measuring method in which the photographic step is omitted. Each curved coleoptile is removed from the plant and placed upon a glass plate over a protractor. The angle is determined indirectly. Navez and Robinson (1932*a*) have described an automatic photographic method.

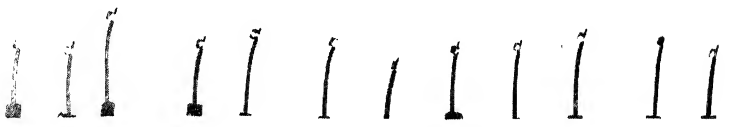


FIG. 15.—Shadow pictures of *Avena* coleoptiles which have curved in response to unilateral application of agar blocks containing growth hormone. These curvatures may be measured with a protractor such as is shown in Fig. 14.

Went (1928*a*) states that curvatures over a range of about 1 to 20 deg. are strictly proportional to the concentration of growth substance in the agar; hence if the mean curvature of 12 plants is 20 deg. or less, an accurate determination of the concentration is possible (Fig. 2, Went, and Fig. 11). If the curvature is much greater than 20 deg. ("maximum angle"), the direct relationship between curvature and concentration no longer exists.

7. METHODS OF EXPRESSING THE RESULTS.—Various units have been proposed by workers using this technique, each based upon the degree of curvature of the Avena coleoptile:

One unit is that quantity of growth substance that has to be present in 1 cc. of solution to give, after mixing with 1 cc. agar, an angle of 1 deg. at a temperature of 25°C. and a relative humidity of 85 to 90 per cent. The blocks are prepared from the larger rectangular agar plates mentioned on page 31, and each has a volume of a little over 10 mm.[3] One block is applied to each of the 12 test coleoptiles. The average curvature of the coleoptiles, in degrees, is multiplied by 12. The product, then, may be expressed as *plant units* (Dolk and Thimann, 1932).

One plant unit is the amount of growth substance applied in one agar block, as above, to give an angle of 1 deg. The growth substance has diffused from a plant part into the agar. These blocks are applied to 12 test plants also, so the average result is multiplied by 12, as in the above. In this case the actual amount of material in each block applied to the plant is but $\frac{1}{200}$ of that present in 1 cc.; hence a *plant unit* is $\frac{1}{200}$ *unit* (Dolk and Thimann, 1932).

One Avena Einheit, or *AE*, is the amount of growth substance present in one block of agar 2 by 2 by 0.5 mm. that will cause a 10 deg. curvature at 22 to 23°C. and at a relative humidity of 92 per cent (Kögl and Haagen Smit, 1931, Mitt. I).

Comparison of the Units of Different Workers.—The relationship of the d value used by Boysen Jensen and curvature as measured in degrees (Went and others) is as follows: If d is the difference in length of the two sides of the curved coleoptile, and φ the angle of curvature (Fig. 16), the relationship between them may be stated in the following way:

$$d \quad \frac{\varphi 2\pi(r+t)}{360} \cdot \frac{\varphi 2\pi r}{360} = \frac{\varphi 2\pi t}{360}$$

With the aid of this equation the difference in length d between the convex and concave sides of the coleoptile can be calculated from the angle of curvature. If the coleoptile is 1.5 mm. thick, then for $d = 1$ mm., φ is equal to an angle of 38.2 deg.; this is much greater than the maximum angle (20 deg.) permissible for quantitative work, but d values of 0 to 0.5 or 0.6 mm. have a direct relationship with the concentration of growth substance.

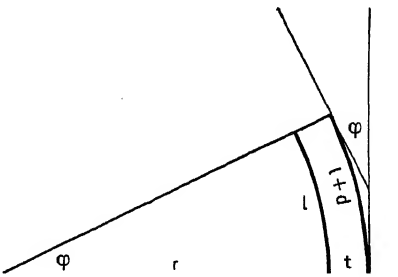


FIG. 16.—Diagram to show relationship of d value (used by Boysen Jensen) to curvature measured in degrees (used by Went and others).

Still another method for determining curvature is given by Metzner (1929).

An exact comparison between the units mentioned above is not possible, because of the difference in the cultural and experimental conditions and the size of the agar blocks employed. However, to get a clearer conception of the relative values of the units, a few equivalents are given (Table 1): they are intended to be little more than suggestive.

It can be demonstrated that 1 mg. pure, crystallized auxin contains 20 to 90 $\times$ 10^6 AE (Kögl, Haagen Smit, and Erxleben, 1933, Mitt. IV); therefore, according to Table 1, this same amount of auxin should be equivalent to 100 to 450 WAE, or 2 to 9 $\times$ 10^5 "units," when applied to Avena coleoptiles.

TABLE 1.—COMPARISON OF THE UNITS USED BY DIFFERENT INVESTIGATORS FOR EXPRESSING GROWTH-SUBSTANCE CONCENTRATIONS

	WAE	Units	AE	Plant units
1 WAE	1	2,000	200,000	400,000
1 unit	0.0005	1	100	200
1 AE	0.000005	0.01	1	2
1 plant unit	0.0000025	0.005	0.5	1

The computations in Table 1 were carried out in the following way: A comparison between the WAE and the AE shows that the former is dissolved in 100 cc., the latter in 2 cc.; since the result in the first case is four times greater than that in the second, one may conclude that 1 WAE corresponds to about 200,000 AE. If the size of the agar block is considered, as is done by duBuy and Nuernbergk (1932), the results are different; they compute that 1 WAE corresponds to approximately 54,000 AE. Since the curvature is not proportional to the size of the agar block, this value is probably too low (the size of the block used by Boysen Jensen is, moreover, 1 by 2 by 2 mm. = 4 $mm.^3$ and not 7.5 $mm.^3$, as stated by duBuy and Nuernbergk).

Other Quantitative Methods. *Growth in Length of Decapitated Coleoptiles.*—If an agar block containing growth substance is placed over the entire cut end of a newly decapitated coleoptile, its rate of growth in length is accelerated, in comparison with the controls (Fig. 1, Went). Although this method can be used as a measure of the growth-substance concentration in the agar block, it is not so accurate as the procedures outlined above.

Pea-test Method.—This means of determining concentrations of the growth hormone in solutions was described by Went (1934*b*). Seeds of *Pisum sativum* are grown in sand cultures in the dark-room until the epicotyl is 5 to 20 cm. in length. Pieces 2 to 20 cm. long are cut from the stem about 5 cm. below the growing point and may be used immediately or in 4 to 8 hours after cutting. Just before immersing the stem segments in the growth-substance solutions of unknown concentration, the distal end of the segment is split lengthwise by an exactly median cut for a distance of 1 to 3 cm. Subsequent immersion in the solutions causes the following: If no growth substance is present, each half will curve outward; if growth substance is present, the free ends will start to

bend inward after about 1 hour of immersion at 25°C. The higher the concentration the greater the bending. The final state of curvature is reached in about 6 hours. In making this test quantitative, the curvatures were evaluated in the following way (Fig. 17):

0 = no auxin reaction, 2 halves concave at the outside over their whole length (see figure).
1 = slight auxin reaction, trace of convexity in limited region.
2 = definite auxin reaction, ends of both halves approximately parallel.
3 = fair auxin reaction, ends bent inward (see figure).
4 = strong auxin reaction, ends just touching.
5 = very strong auxin reaction, both halves crossing at end.
6 = exceptionally strong auxin reaction, halves crossing midway or at base (see figure).

Callus-forming Effect of Growth-substance Pastes.—Laibach and Fischnich (1935*a*) used *Vicia Faba* as test plants. The seeds

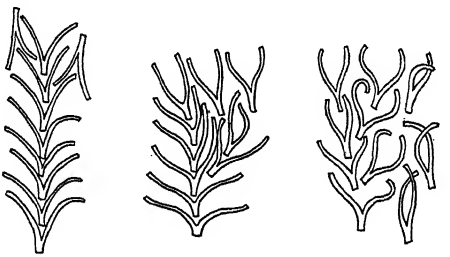


FIG. 17.—Quantitative method (approximate) for measuring the concentration of growth hormone present in solutions. Split sections of pea stems when immersed in growth-hormone solutions exhibit a certain amount of curvature depending upon the concentration of the active substance present. *A* represents their appearance when immersed in water; *B* in a solution containing 30 units per cc.; and *C* in a solution containing 150 units per cc. (*After Went*, 1934*b*.)

are soaked overnight and then planted in 7 cm. pots in coarse sand; they are kept in a greenhouse at a temperature of 25°C. After 10 days the epicotyl is 20 to 25 cm. long, and the plants are ready to use. They are decapitated just below the second node on the tenth day and moved into the darkroom until the end of the experiment. Here the temperature is maintained at 23°C., and the humidity at 70 per cent.

To determine the concentration of a growth-substance solution, it is mixed with lanolin paste and applied to the cut end of a decapitated epicotyl. Increase in cross-sectional thickness of the

stump is measured after 4 days and is expressed in percentage of the original thickness.

A *Vicia unit* of effect is defined as that degree of callus formation after 4 days which corresponds to a 10 per cent increase in thickness of a decapitated epicotyl of Vicia. The Vicia unit corresponds to the effect of 73γ 3-indole acetic acid in 1 g. of paste.[1] The concentration of the unknown paste can be determined by comparing its callus-forming effect with the standard

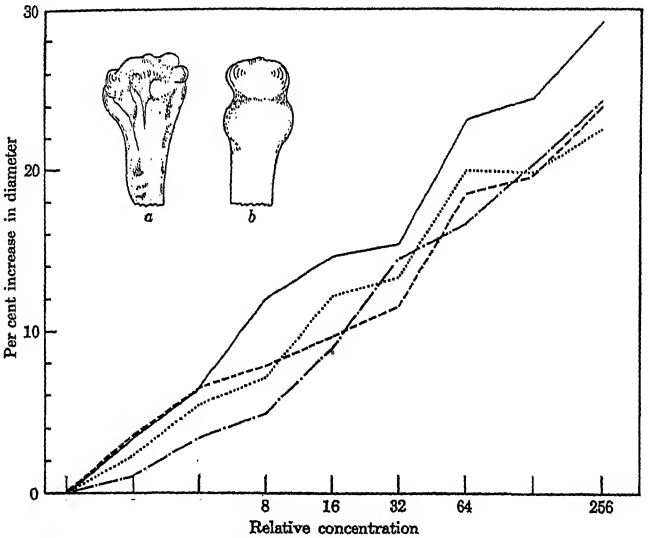


FIG. 18A.—Relationship between concentration of 3-indole acetic acid applied (in lanolin) to epicotyls of *Vicia Faba*, and per cent increase in their diameter; *a* of the inset shows the appearance of a swelling caused by a dilute application of growth hormone to the cut apical surface of a seedling stem; *b*, the effect of a strong application. (*After Laibach and Fischnich*, 1935*a*, *and Laibach*, 1935.)

curve (Fig. 18A). About 50 plants should be used for a single test.

The accompanying standard curve was obtained from observations on three series of plants, 280 plants in each series. According to the graph published by Laibach and Fischnich, the direct relationship between growth-substance concentration of the paste and increase in thickness holds up to about 3 Vicia units, or 30 per cent increase in thickness of the stump due to callus formation.

Comparison of Avena with other Test Objects.—The Avena coleoptile has proved to be a successful test object in most

[1] One gamma (γ) = $\frac{1}{1000}$ milligram.

instances, but in certain cases it has failed to show a response. Stark (1921*a*) found that segments of the shoot of *Brassica napus* placed on one side of a decapitated Avena coleoptile caused positive curvature, toward the side with the applied object, thus indicating inhibition of growth. Gradmann (1928) obtained similar results using tips of Convolvulus. In a more extensive study Söding (1935*b*) found that certain genera, such as Symphoricarpos, Rheum, and Cephalaria, do not give the Avena coleoptile test for growth substance. However, when decapitated seedlings of the hybrid *Cephalaria tatarica* × *C. alpina* were employed as test objects, positive indication of growth-promoting substance was obtained by unilateral application of agar blocks containing exudate from seedlings or parts of mature plants of *C. tatarica*. This suggested that there might be other types of growth hormone in plants distinct from those in Avena. Also, there is the possibility of the existence of toxic substances or destructive enzymes in the growth-substance test preparations which nullify the growth-promoting effects. Moreover, the failure of growth hormone from one plant source to penetrate the tissue of another plant used as the test object could explain the failure to get response. Recently, however, Söding has shown that blocks containing hormone from Avena give test curvatures with Cephalaria (1935*c*). Söding (1936) has pointed out, furthermore, that in many instances Cephalaria is more sensitive than Avena. For example, in testing the growth-substance content of Taxus, about 15 times greater curvatures were obtained with Cephalaria. Söding concluded that the difference in response of test plants is due, not to the existence of chemically different growth substances in different plants, but rather to the difference in sensitivity to the presence of low concentrations of the same hormone. Experimental evidence was presented to show that Avena is relatively unresponsive to extremely dilute preparations of heteroauxin while Cephalaria exhibits marked curvatures to the same low concentrations of the hormone; on the other hand, the amount of curvature shown by Avena over the range in which growth is proportional to concentration of hormone indicates its superiority for general quantitative test purposes (Fig. 18*B*). In order to increase sensitivity in Avena as a test object, Heyn (1935) has resorted to three decapitations, allowing two hours between each, and

having the last one come just before the agar blocks are to be applied.

Kornmann (1935) found that a bending response could be obtained with extracts from corn meal or from tips of maize coleoptiles applied in agar blocks to Avena coleoptiles. There was no response if Avena-coleoptile tips or bases were placed upon these blocks for a few hours before testing. Some substance diffusing from the Avena tips into the agar inactivated the maize-agar preparation. When maize coleoptile tips were

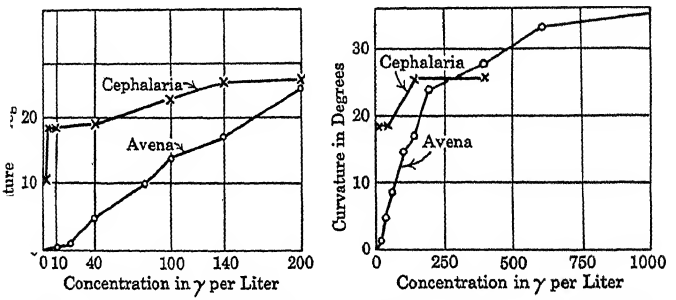


FIG. 18B.—Graphs showing growth curvatures in *Avena* and *Cephalaria* when treated with different concentrations of heteroauxin (3-indole acetic acid). Left: *Cephalaria* shows greater sensitivity to low concentrations of growth hormone. Right: same, over greater range of concentrations (*From Söding*, 1936.)

placed on corn-paste agar, better curvatures resulted than with the corn-paste alone. Similarly, good curvatures resulted with oat-flakes paste upon which Avena coleoptile tips had stood. No curvature followed the application of a mixture of maize-tip paste and oat-tip paste, each of which alone gave good results. The suggestion is made that in certain of the mixed preparations, destructive oxidation of the hormones resulted from the action of enzymes.

SUMMARY

It has been demonstrated beyond question that growth substances occur in plants, although they are present in such small quantities that no satisfactory means of microchemical detection has yet been devised. The most commonly used biological indicator is the Avena coleoptile.

Seedlings of Avena which are to be used in testing for the presence of a phytohormone are usually grown in a darkroom

under controlled conditions, *e.g.*, at a temperature of 25°C. and 90 per cent relative humidity. The procedure ordinarily is carried out in phototropically inactive light.

When the coleoptiles have attained a length of 25 to 40 mm., the tip is removed, and the object to be tested is placed on one side of the cut surface of the coleoptile stump. Small portions of plant organs may be tested for the presence of growth substance by applying them directly to the decapitated coleoptiles, or they may be placed on moist agar into which the hormone will diffuse from the plant part. The agar is then cut into standard-sized blocks and applied unilaterally to the decapitated coleoptiles.

If a minute amount of a growth hormone is present in the object to be tested, it brings about increased growth on the side of the coleoptile to which it is applied, thus producing a curvature; this curvature may be taken as an indication of the presence of a growth hormone. Curvatures, over a certain range, are proportional to the concentration of the hormone present in the object being tested. It is upon this fact that quantitative determinations depend.

For purposes of demonstration, the coleoptile tips removed by decapitation may be replaced on one side of the cut surface with a little gelatin. Curvature will follow. Numerous other plants have been used in diverse ways for demonstrating the presence of growth substances.

CHAPTER III

PREPARATION AND PROPERTIES OF GROWTH SUBSTANCES

Growth substances have been prepared in large amounts from both plant and animal materials. Fungus cultures were used as a source by Nielsen and Boysen Jensen and later by Thimann and others. Human urine was discovered by Kögl and his associates to be a rich source of substances that promote growth in plants. Later it was discovered that maize oil, malt, and yeast are fairly rich sources, and, still more recently, numerous synthetic compounds have been found to be quite active in promoting growth. It remains to be shown whether the latter act as true growth-promoting substances or merely influence hormones naturally present in the plant.

Kögl has proposed the inclusive term *auxin* for growth substances that bring about cell enlargement; three such auxins have been isolated in pure crystalline form from plant materials. The chemical name auxin and the physiological name *growth substance* are equally useful and interchangeable terms.

PREPARATION FROM PLANT AND ANIMAL SOURCES

Rhizopus, a Source of Growth Substance.—Nielsen (1930*a*, *b*) first showed that growth substance could be extracted from the substratum on which either *Rhizopus suinus* or *Absidia ramosa* was cultured. Growth substance was formed when Rhizopus was cultured only on a solid medium. His method follows:

(1) Petri dishes with a diameter of 18 cm. were used, and two pieces of filter paper were placed in each. (2) The dishes were filled with a solution made up of 10 g. glucose, 10 g. ammonium tartrate, 0.5 g. monobasic potassium phosphate, 0.5 g. magnesium sulphate, 10 drops 1 per cent ferric chloride, and 1,000 cc. water. (3) The fungus spores were planted and grown at 35°C. for 6 days. (4) The fluid substrate together with the liquid expressed from the

filter paper contained the growth substance, which Nielsen named *rhizopin*. (5) Rhizopin is soluble in water, alcohol, 90 per cent acetone, and ether. It can be extracted from aqueous solution with ether but not with xylene or benzene. It is very sensitive to peroxide.

The ether should be freed from peroxides before use. Its purification may be accomplished by the Garbarini method, as follows: 2 liters of ether are shaken up with 1 g. calcium hydroxide and 3 g. ferrous sulphate in 20 cc. water. The ether is then distilled. Rhizopin soon becomes ineffective if dissolved in ether that has not been purified in some such way as this.

The Identity of Rhizopin and 3-Indole Acetic Acid.—Growth substance prepared from Rhizopus and Aspergillus was shown (Kögl and Kostermans, 1934, Mitt. XIII) to have a molecular weight close to that of 3-indole acetic acid, and Thimann (1935*b*) showed that the active substance "rhizopin" is almost certainly identical with 3-indole acetic acid; in the same paper, Thimann outlined the steps for purification of this substance extracted from Rhizopus.

Aspergillus, a Source of Growth Substance.—Boysen Jensen (1931*b*) showed that *Aspergillus niger* forms growth substance when cultured on a fluid substratum, but only when peptone or haemoglobin is present as a source of nitrogen. The method of preparation was as follows:

(1) Large covered culture dishes of tin plate, 37 by 58 cm., were used for growing the fungus. The dishes were coated on the inside with a solution of paraffin in petroleum ether. (2) Each culture vessel contained about 3 liters glucose-peptone solution (1.5 cm. in depth). Each liter of solution contained 20 g. impure glucose, 5 g. peptone, 5 g. citric acid, and 0.25 g. monobasic potassium phosphate added to distilled water. (3) A layer (2 to 5 mm. in depth) of sterilized cork particles was placed on the culture fluid to form a substratum for the fungus. (4) The culture medium was inoculated with Aspergillus spores which had been soaked in sterilized water. (5) The temperature was maintained for 3 days at 33 to 34°C. If the culture showed no infection at the end of this time, the temperature was raised to 36 to 37°C. for the next 7 days. The production of growth substance apparently takes place chiefly after the cessation of growth. When the reaction of the culture fluid becomes alkaline, the

maximum concentration generally has been reached, *i.e.*, as much as 400 WAE per liter.

The method of concentration was as follows:

(1) The culture fluid was filtered into portions of 10 liters, acidified with citric acid and concentrated under reduced pressure to 200 cc. Concentration also may be carried out in large enameled dishes at 50 to 60°C. Growth substance is fairly stable in the impure condition. (2) The concentrated filtrate was again filtered and extracted with an equal amount of peroxide-free ether for 24 hours, being rotated slowly; this process usually was repeated four times. (3) The purified ether extracts were evaporated over 100 cc. distilled water. (4) The aqueous solution was neutralized by the addition of 2 g. sodium bicarbonate and shaken out three times with 100 cc. peroxide-free ether, to remove various ether-soluble compounds. The growth substance, which is an acid, remained in the aqueous solution. (5) The aqueous solution was acidified with citric acid and shaken out three times with 100 cc. peroxide-free ether. (6) The ether extract was evaporated, and the residue dried over calcium chloride in a vacuum desiccator. The residue was extracted by boiling petroleum ether for 20 minutes. (7) The growth substance did not pass over into the petroleum ether, and after the latter was evaporated off it was extracted from the residue by treatment with 100 cc. cold water for one hour. (8) The growth-substance solution thus obtained contained some citric acid, which was removed by neutralization with sodium bicarbonate, weak acidification with acetic acid, and extraction three times with 100 cc. peroxide-free ether. (9) The ether was distilled over 100 cc. distilled water, and the growth substance thus was obtained in aqueous extract. This was kept at low temperatures.

Purification entailed appreciable losses of growth substances; if there were 2,000 WAE present in the culture fluid after the original concentration (step 1), only about 800 to 1,000 WAE remained in the purified solution. One milligram of the dry substance contained 4 to 10 WAE, and the product was sufficiently pure for physiological investigations.

Substratum Content and Growth-substance Production.—Boysen Jensen (1932) subsequently found that Aspergillus could convert tryptophane and other amino acids (lysine, leucine, tyrosine,

and phenylalanine) into growth substance (see discussion on formation).

Urine, a Source of Auxentriolic Acid (Auxin a).—The following outline of procedure is that of Kögl, Haagen Smit, and Erxleben (1933, Mitt. IV). It may be followed with reference to Table 2.

TABLE 2.—CONCENTRATION AND PHYSIOLOGICAL ACTIVITY OF GROWTH HORMONE AT DIFFERENT STAGES OF ITS PREPARATION FROM URINE

Stage	Weight, grams	AE per milligram	Total content, millions of AE
Urine concentrate	5,700	1,840	10,488
After extraction with ether	87	119,600	10,405
After fractionation with bicarbonate solution	45	143,520	6,458
After extraction with petroleum ether and ligroin	19.7	437,920	8,627
After purification with benzene	5.5	1,104,000	6,072
After lead-salt precipitation	3.17	2,024,000	6,416
After calcium-salt precipitation	2.25	2,944,000	6,624
After esterification or lactone formation.	1.2	4,600,000	5,520
After high-vacuum distillation	0.179	9,200,000	1,647
Crude crystallate	0.0397	21,000,000	840
After recrystallization		50,000,000	

1. *Concentration of the Urine.*—One hundred and fifty liters of mixed urine (auxin content, 300 mg.) were acidified with hydrochloric acid (1:1) until the reaction was acid to Congo red. The fluid was then concentrated in batches of 30 liters in a distilling apparatus until it became a thick, dark-brown, partially crystallized syrup. The residues of the five batches totaled 5,700 g. in weight, and these were combined for further steps in purification (see Table 2).

2. *Ether Extraction of the Crude Syrup.*—The crude syrup was dissolved in 25 to 30 liters of water, acidified with hydrochloric acid, and repeatedly shaken with an equal volume of purified peroxide-free ether. The ether extracts were combined and dried over sodium sulphate, then concentrated (boiled down). The residue of 87 g. contained almost all the active substance concentrated sixty-five times.

3. *Fractionation with Sodium Bicarbonate Solution.*—The ether residue was dissolved again in the smallest possible amount of pure ether (1 to 3 liters) and shaken up eight times, each time with 500 cc. saturated bicarbonate solution. Dilute hydrochloric acid was added to the combined bicarbonate extracts, and the acid solution was extracted with ether six to eight times; 3 to 4 liters of ether were necessary for this step. The ether was dried over anhydrous sodium sulphate and was evaporated down. A residue weighing 45 g. was obtained. The active substance at this point had been concentrated seventy-eight times.

4. *Extraction with Petroleum Ether and Ligroin to Remove More Inert Material.*—The ether residue was heated for ½ hour on a water bath with 400 cc. petroleum ether (b.p. 40 to 60°C.). After cooling, the solution was carefully poured off, and the extraction was repeated twice in the same way with fresh petroleum ether; the physiologically ineffective substances went into solution in the petroleum ether and were removed in this way. The auxin remaining in the syrup-like material was insoluble in petroleum ether. This syrup was extracted three times, each time by heating the residue with 400 cc. ligroin (b.p. 100 to 120°C.). The residue weighed 19.7 g., and the active substance was 238 times more concentrated than in the original material.

5. *Extraction with Benzene.*—The syrup thus far purified was dissolved in 300 cc. 60 per cent ethyl alcohol and shaken ten successive times, each time with a fresh 100 cc. portion of benzene. The combined benzene solutions were extracted three times with water, using 300 cc. each time, then with 50 per cent methyl alcohol three times, using 300 cc. each time. The methyl alcoholic extracts were evaporated to dryness; the residues from the methyl alcoholic and the aqueous extracts were combined, and this was shaken several times with ether. After the ether was dried and evaporated, the residue weighed 5.5 g. The active substance was then six hundred times concentrated.

6. *Lead-salt Precipitation.*—The ether residue was dissolved in 125 to 150 cc. 96 per cent alcohol and mixed with a concentrated aqueous solution of 5 g. neutral lead acetate. The resulting precipitate contained almost no auxin. To the filtrate was added drop by drop a 30 per cent sodium hydroxide solution until a weak alkaline reaction was obtained. The precipitate thus formed was filtered off, dissolved in dilute acetic acid, and

the acid solution extracted with ether. The amount of residue fluctuated greatly, as did the content of physiologically active substance. In several cases more than 80 per cent of the auxin was present in the precipitate in the alkaline medium, so that this was important in the further steps of preparation. When only 40 to 60 per cent of the active substance previously present was in the precipitate, the filtrate was acidified with glacial acetic acid, concentrated until removal of the alcohol, then extracted with ether. The ether residue containing the remaining amount of active substance was further treated, either alone or together with the precipitate fraction (above mentioned). In most cases the greater amount of the active substance remained in the filtrate so that the precipitate could be disregarded. The remaining portion of the active fraction weighed 3.17 g. (1.08 to 7.6 g.). At this point, usually about 60 per cent of the active substance originally present in the urine was still present; it had been concentrated eleven hundred times.

7. *Calcium-salt Precipitation.*—The most active residue from the lead-salt fractionation, usually about 3 g., was dissolved in 30 cc. alcohol, and 300 cc. water were added; at this point, the solution became slightly turbid. A concentrated aqueous solution of 6 g. calcium acetate was added to this solution, and then a normal solution of potassium hydroxide was added during frequent shakings until no further precipitation took place. The precipitate was filtered off and repeatedly washed with aqueous alcohol. The resulting filtrate was acidified with glacial acetic acid and extracted with ether. The ether was evaporated, and the ether residue weighed 2.25 g. The active substance was sixteen hundred times more concentrated than in the original material.

8. *Lactone Formation.*—The clear, reddish-brown syrup was boiled for one hour with 10 to 15 cc. 1.5 per cent methyl alcohol in hydrochloric acid. After removal of the alcohol in a vacuum the residue was taken up in ether, and the ether solution shaken up twice with 2 per cent sodium bicarbonate solution and twice with water. The auxin remained in the neutral fraction (the ether solution). The ether solution was evaporated, and the residue weighed 1.2 g. (0.47 to 2.45 g.). At this point, the active substance was 2,500 times more concentrated than in the original material.

9. *High-vacuum Distillation.*—The concentrated syrup was distilled as slowly as possible in portions of 400 to 800 mg. in a high vacuum (at 0.005 to 0.02 mm. mercury). As a rule, four or five fractions were obtained. (*a*) The first distillate which passed over at the temperature of the bath (below 125°C.) had an average effectiveness of 2,500,000 AE per milligram and gave no crystals of the active auxin. (*b*) The distillates that passed over at 125 to 135°C. contained the largest part of the active substance and showed an average effectiveness of 9,200,000 AE per milligram (3,100,000 to 24,000,000 AE). In these middle fractions, active crystals separated out after remaining several days in the refrigerator. Occasionally it was necessary to cool with an acetone-carbonic acid mixture; these crystals were filtered off and could be purified further. (*c*) At a water-bath temperature of 135 to 150°C., very active fractions sometimes were obtained (the values fluctuated between 120,000 and 23,700,000 AE per milligram); these formed crystals. The residue remaining had an average effectiveness of 1,100,000 AE per milligram. The amount of the active middle fractions averaged 179 mg. (89 to 336 mg.). The auxin at this point was five thousand times concentrated.

10. *Purification of the Crude Crystallates.*—The syrup containing active crystals (the middle fraction) was siphoned off; the crude crystals had an effectiveness of 21,000,000 AE per milligram. They could be purified further by recrystallization.

Six recrystallizations with a mixture of ligroin and ethyl alcohol (1:1) yielded crystals with a melting point of 196°C. and an average effectiveness of nearly 50,000,000 AE per milligram. These crystals were auxin *a*.

Four recrystallizations with 40 per cent acetone gave crystals with a melting point of 173°C. and an average effectiveness of about 35,000,000 AE per milligram. The crystals obtained in this way were the lactone of auxin *a* [the designation auxin *a* was not introduced until auxin *b* was discovered (Kögl, Haagen Smit, and Erxleben, 1933, Mitt. VII)].

Maize Oil, Malt, and Other Sources of Auxenolonic Acid (Auxin b).—Both auxin *a* and auxin *b* have been prepared from maize oil and malt (Kögl, 1933; Mitt. VI; Kögl, Haagen Smit, and Erxleben, 1933, Mitt. VII; Kögl, Erxleben, and Haagen Smit, 1934, Mitt. IX; Kögl and Erxleben, 1934, Mitt. X), also

from peanut, sunflower, mustard, and linseed oils (Mitt. IX, 1934). Preliminary tests indicated that maize oil is rich in growth substance. Preparation of the auxin was started with 16 kg. maize oil, divided into separate 1 to 2 kg. portions. Each portion was mixed with twice its volume of water and shaken for 3 hours, then centrifuged. The aqueous solution was acidified with hydrochloric acid and then extracted with ether. The procedure from this point on is essentially the same as that for obtaining auxin from urine. After auxin *a* was removed, the more active fractions that boiled at lower temperatures yielded crystals with a melting point of 183°C. (auxin *b*) in contrast to 196° for auxin *a*.

Auxin *b* has an average physiological effectiveness of 50,000,000 AE per milligram.

Urine, a Source of 3-indole Acetic Acid (Heteroauxin).—Kögl, Haagen Smit, and Erxleben (1934, Mitt. XI) prepared another growth substance from urine which they called *heteroauxin.* It is 3-indole acetic acid and had been isolated previously (Salkowski, 1885) from normal and pathological urines. It has been prepared synthetically by Majima and Hoshino (1925).

The total growth-substance content of urine is about 80 per cent auxin *a* and 20 per cent 3-indole acetic acid (Kögl, Haagen Smit, and Erxleben, 1934, Mitt. XI). The physiological activity of the latter is approximately 25,000,000 AE per milligram. It was prepared from urine in the following way:

(1) One kilogram decolorizing carbon was mixed with 100 liters urine and allowed to stand overnight. The charcoal then had settled so that the fluid could be siphoned off. The charcoal was drained and washed with water. (2) Next it was mixed with 2 liters aqueous acetone ammonia (60 per cent acetone, 5 per cent ammonia); this carbon infusion was filtered off quickly, and a light yellow eluate remained. (3) Five liters of acetone ammonia (60 per cent acetone, 2.5 per cent concentrated ammonia) were siphoned through (preferably overnight). The entire eluate was stirred thoroughly with an excess of ammonium sulphate, until the fluid had divided into two layers of about the same depth. The upper auxin-containing layer was siphoned off and distilled. The last remains of the acetone were removed in a vacuum. (4) The residue was dissolved in water and extracted with ether, the ether residue boiled in petroleum ether

and ligroin, and the insoluble portion fractionated over sodium bicarbonate. (5) The residue of the acid fraction was dissolved in 60 per cent ethyl alcohol and shaken up with benzene. The active substances were extracted from the benzene solutions with 50 per cent methanol and finally with water. After removal of the alcohol, the aqueous solution was extracted with ether, and the residue again treated with petroleum ether and ligroin as above. (6) The portion insoluble in ligroin was heated on a water bath six to eight times, each time with 50 to 100 cc. xylene; the growth substance went into solution almost entirely. (7) After removal of the xylene, the residue was extracted about six times on the water bath, each time with 50 to 80 cc. cyclohexane; the active substance then remained in the insoluble residue. The effectiveness after this step was (at most) 2,000,000 AE per milligram. (8) After treatment with cyclohexane, lead-salt fractionation was carried out. The precipitate, in a weakly acid medium, was discarded; determinations on the filtrate showed it to have an effectiveness of about 3,000,000 to 6,000,000 AE per milligram. (9) The usual calcium-salt precipitation followed, from which only the filtrate was treated further. (10) The growth-substance concentration in the filtrate was only slightly greater than in the previous step, so that the highly active product (about 1.5 g.) was dissolved next in 18 cc. alcohol and mixed with 180 cc. water; 5 g. barium acetate in saturated aqueous solution was added. (11) Then normal potassium hydroxide solution was added until no more precipitate formed. The precipitate was entirely inactive; the filtrate, after decomposition with acid, showed an effectiveness of about 15,000,000 AE per milligram. Crystals formed in this residue after it had remained in the refrigerator for 2 days. These crystals were filtered off and washed with a little cold chloroform. The crude product of about 135 mg. had a melting point of 148 to 156°C. (12) This product was recrystallized from chloroform and yielded 111 mg. The crystals were rhombic plates with a melting point of 161 to 162.5°C. and an effectiveness of 20,000,000 AE per milligram.

These rhombic plates were three times recrystallized from chloroform, giving 100, 91, and 82 mg., respectively. The melting point remained constant at 164°C.

Another quantity of material was prepared in the same way as the foregoing and eventually recrystallized four times from

chloroform and twice from water. These crystals had a constant melting point of 165°C. and showed the same strength in AE per milligram as the first.

Yeast, a Source of Heteroauxin.—Kögl and Kostermans (1934, Mitt. XIII) have isolated partially pure heteroauxin from cultures of yeast, after plasmolysis by ammonium chloride. The melting point of the preparation was 163.5°C., and its molecular weight 193. Its physiological effectiveness amounted to 17,600,000 AE per milligram. Further purification might be expected to bring it still closer to the characteristics of 3-indole acetic acid, both in melting point and in physiological effectiveness. Similar preparations of heteroauxin from Rhizopus and Aspergillus showed molecular weights of 176 and 169, respectively, in good agreement with the molecular weight of 175 for 3-indole acetic acid.

The method employed by Kögl and Kostermans in the preparation of the growth substance from yeast was as follows:

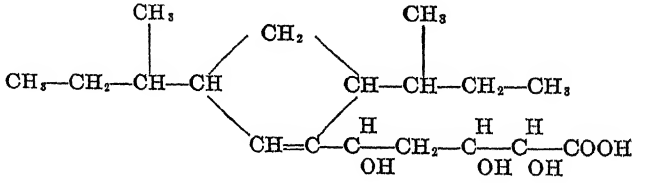
(1) One kilogram finely crushed yeast was mixed with 100 g. pulverized ammonium chloride and allowed to stand for 24 hours. This gave a viscous mass, which was acidified with hydrochloric acid and extracted directly with a large quantity of ether, since it was found difficult to filter or centrifuge it without great losses. Two and one-half liters of peroxide-free ether per kilogram of yeast were used for the ether extraction; in this step, shaking is done with care in order to avoid the formation of inseparable emulsions. In this way, a total of 50 kg. of baker's yeast was plasmolyzed with ammonium chloride and extracted with ether. (2) The ether extract was evaporated down to 5 liters and shaken up with 5 per cent bicarbonate solution. After evaporation of the ether, a syrup with an unpleasant fatty-acid odor remained. (3) For the purpose of removing the lower fatty acids, the mixture was heated in a vacuum to 100°C. (water-bath temperature). There remained a residue of 6.5 g. with an effectiveness of 120,000 AE per milligram. A small sample of this material mixed with ferric-chloride-hydrochloric acid gave the red color reaction for 3-indole acetic acid; after each of the further steps, this color reaction was used to indicate whether or not the greater amount of growth substance was still contained in the expected fraction. (4) The active syrup was boiled three times with benzene; the insoluble residue was then 3.5 g., with an effectiveness of 250,000 AE per milligram. (5)

After the usual fractionation with benzene the active syrup was heated eight times with xylene at water-bath temperature; the xylene extracts were combined and evaporated to dryness in a vacuum. The residue (500 mg.) had an effectiveness of 1,200,000 AE per milligram and contained about 20 mg. of 3-indole acetic acid. Crystals formed in this crude oil after it had been in the refrigerator for a day; these were siphoned off and washed several times with a little cold chloroform. The color reaction of this crude crystallate with ferric chloride-hydrochloric acid was intense, but the effectiveness amounted to only about 2,000,000 AE per milligram. (6) Recrystallization from chloroform increased the purity and physiological activity too slowly, so the crystallate was boiled twice with benzene, and the benzene solutions discarded. Then the insoluble matter was recrystallized twice from water. There resulted 9 mg. of a product with a melting point of 163.5°C. and an effectiveness of 17,600,000 AE per milligram.

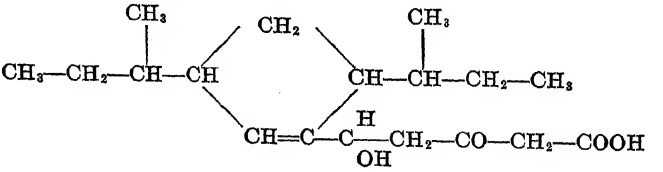
PROPERTIES OF GROWTH SUBSTANCES

The Structural Constitution of the Auxins.—The structure of the foregoing auxins is now well-known (Kögl, 1935, Mitt. XIV). They are all monobasic acids and have one double bond. Auxin *a* forms a lactone with the same empirical formula as auxin *b*.

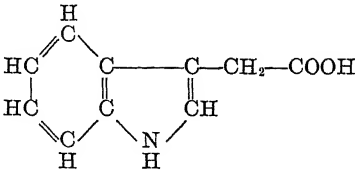
Auxentriolic acid (auxin *a*) is a monocyclic trihydroxycarboxylic acid, with one double bond, and has the structural formula



Auxenolonic acid (auxin *b*) is a monocyclic hydroxyketocarboxylic acid, with one double bond, and has the structural formula



β-Indole acetic acid, or 3-indole acetic acid, heteroauxin, has the structural formula



For physical and chemical characteristics of the foregoing, see the accompanying list.

Physical and Chemical Characteristics of the Auxins Crystallized from Plant Materials.—This list is a compilation from various studies in the Kögl, Haagen Smit, and Erxleben series. Reference is made in roman numerals to the Mitteilung number.

Characteristics	Auxentriolic acid (auxin *a*)	Auxenolonic acid (auxin *b*)	3-Indole acetic acid (heteroauxin)
Empirical formula..	$C_{18}H_{32}O_5$ (V)	$C_{18}H_{30}O_4$ (IX)	$C_{10}H_9O_2N$ (XI)
Molecular weight...	328 (V)	310 (IX)	175 (XI)
Crystal characteristics and melting point.	Colorless, hexagonal crystals 196° (V)	183° (IX)	164–165° (XI)
Dissociation constant.....	$K = 1 \cdot 10^{-5}$ (XVI)		
Specific gravity.....	$s = 1.292$ at 19° (IX)	$s = 1.269$ at 20° (IX)	
Specific rotation....	$(a)_D^{20} = -3.19°$ (V)	$(a)_D^{20} = -2.79°$ (IX)	$(a)_D^{20} = -3.8°$ (XI)
Solubilities.....	Readily soluble at low temperatures in methanol, ethanol, and ethyl acetate; not readily soluble in ether; ca. 1% soluble in cold water; practically insoluble in petroleum ether, ligroin, benzene (V)	Soluble in ether, sensitive to peroxide (IX)	
Other characteristics	Thermostable; not decomposed by light; becomes physiologically inactive as a growth promoter after a few months, by isomerization, even if kept under vacuum in the dark (V)	Isomer of auxin *a* lactone; light- and heat-stable; crystals lose activity in a few months by isomerization; easily oxidized; first prepared from corn-germ oil (IX)	Probably produced by microorganisms (XIII)
Acid and alkali sensitivity.	Stable to acid; sensitive to alkali (XII)	Sensitive to acid; sensitive to alkali (XII)	Sensitive to acid; stable to alkali (XII)

Physiological Effectiveness of the Auxins, Their Derivatives, and Other Compounds.—All the following compounds have been reported on by Kögl (1935, Mitt. XIV) and by Kögl and Kostermans (1935, Mitt. XVI). Activity is indicated in the list presented below in terms of the Avena-curvature test.

Compound tested	Formula	Effectiveness, AE per milligram
Auxentriolic acid (auxin *a*)	$C_{18}H_{32}O_5$	50,000,000
Auxin-*a* lactone	$C_{18}H_{30}O_4$	35,000,000
Auxin methyl ester	$C_{19}H_{34}O_5$	Inactive
Dihydroauxin	$C_{18}H_{34}O_5$	Inactive
Dihydroauxin lactone	$C_{18}H_{32}O_4$	Inactive
Auxin-*p*-phenylphenacyl ester	$C_{32}H_{42}O_6$	Inactive
Tri-*m*-dinitrobenzoyl auxin	$C_{39}H_{38}O_{20}N_6$	Inactive
Auxenolonic acid (auxin *b*)	$C_{18}H_{30}O_4$	50,000,000
Auxin-*b* *p*-phenylphenacyl ester	$C_{32}H_{40}O_5$	Inactive
Auxin-*b* semicarbazone	$C_{19}H_{33}O_4N_3$	Inactive
Lactone of auxin-*b* dimethyl acetal	$C_{20}H_{34}O_4$	Inactive
Dihydroauxin *b*	$C_{18}H_{34}O_4$	Inactive
Monodinitrobenzoyl auxin *b* (hydrated)	$C_{25}H_{32}O_9N_2$	Inactive
3-Indole acetic acid (heteroauxin)	$C_{10}H_9O_2N$	25,000,000
Heteroauxin methyl ester	$C_{11}H_{11}O_2N$	10,000,000
Heteroauxin ethyl ester	$C_{12}H_{13}O_2N$	3,000,000
Heteroauxin *n*-propyl ester	$C_{13}H_{15}O_2N$	1,000,000
Heteroauxin isopropyl ester	$C_{13}H_{15}O_2N$	100,000
2, 3-Dihydro-3-indole acetic acid	$C_{10}H_{11}O_2N$	Inactive
Methyl 2, 3-dihydro-3-indole acetate (methyl ester of the above)	$C_{11}H_{13}O_2N$	Inactive
1-Methyl-3-indole acetic acid	$C_{11}H_{11}O_2N$	30,000[1]
Ethyl-1-methyl-3-indole acetate (ethyl ester of the above)	$C_{13}H_{15}O_2N$	Inactive
2-Methyl-3-indole acetic acid	$C_{11}H_{11}O_2N$	125,000[2]
Methyl 2-methyl-3-indole acetate (methyl ester of the above)	$C_{12}H_{13}O_2N$	Inactive
Ethyl-3-indole acetic ac i..	$C_{12}H_{13}O_2N$	Inactive
5-Methyl-3-indole acetic acid	$C_{11}H_{11}O_2N$	1,500,000
Methyl 5-methyl-3-indole acetate (methyl ester of the above)	$C_{12}H_{13}O_2N$	1,200,000
2, 5-Dimethyl-3-indole acetic acid	$C_{12}H_{13}O_2N$	Inactive[1]
β-3-Indole propionic acid	$C_{11}H_{11}O_2N$	Inactive
3-Indole carboxylic acid	$C_9H_7O_2N$	Inactive
2-Indole carboxylic acid	$C_9H_7O_2N$	Inactive
α-3-Indole propionic acid	$C_{11}H_{11}O_2N$	5,000,000
α-3-Indole lactic acid	$C_{11}H_{11}O_3N$	Inactive
3-Indole-pyroracemic (pyruvic) acid	$C_{11}H_9O_3N$	200,000
β-3-Indole α-aminopropionic acid (tryptophane)	$C_{11}H_{12}N_2O_2$	Inactive

[1] Haagen Smit and Went (1935) report these two compounds equally active (0.002 : active as 3-indole acetic acid). The curvature is confined to a short region at the tip.

[2] Haagen Smit and Went (1935) report this compound only half as active as 1-methyl-3-indole acetic acid. The curvature is confined to a short region at the tip.

Skatole (3-methyl indole) has been reported by Glover (1936) to be active in the Avena test.

Zimmerman and Wilcoxon (1935) have tested several compounds for their ability to cause bending of the stems of the sweet pea and other plants. Not all of these compounds have yet been tested on the Avena coleoptile.

Compound Tested (Sweet-pea Stem Test)	Threshold Value, Percentage in Lanolin
3-Indole acetic acid	0.0005
α-Naphthalene acetic acid	0.05
β-Naphthalene acetic acid	1.00
Acenaphthyl-5-acetic acid	0.05
γ-3-Indole butyric acid	0.025
γ-2-Carboxy-3-indole butyric acid	
Indole propionic acid[1]	0.025
Phenylacetic acid	0.25
Fluorene acetic acid	0.1
Anthracene acetic acid	1.0
α-Naphthyl acetonitrile	0.05

[1] Seven different indole propionic acids were tested; the authors do not state whether these gave different physiological responses.

The same compounds also induce rooting responses in stems of Nicotiana, Lycopersicon, etc., when applied in solution to the soil, injected into the stem, or applied in lanolin directly on the stems. Ethylene and propylene are reported effective when applied in lanolin, and carbon monoxide and ethylene induce roots to grow on stems of numerous species (Zimmerman, Crocker, and Hitchcock, 1933*a*, *b*; Zimmerman and Hitchcock, 1933).

Bauguess (1935) has tested several indole derivatives, with resulting root initiation, stem bending, and bud inhibition in tomatoes, marigolds, and stocks; they are β-3-indole propionic acid, β-4-indole butyric acid, β-3-indole pyruvic acid, β-3-indole α-oximino propionic acid and β-3-indole acrylic acid, all of which are active. The only inactive compound tested was dl-β-3-indole lactic acid.

Haagen Smit and Went (1935) have reported on the activity of the following additional compounds. If the effectiveness of 3-indole acetic acid is considered as 1, the values are as follows:

Compound	Avena Test
3-Indole pyruvic acid	0.2
2-Methyl-3-indole methyl acetate	Inactive
Phenylacetic acid	0.0002[1]
Phenylpropionic acid	Inactive
Cinnamic acid	Inactive
Allocinnamic acid	0.0006[1]
Cis-*o*-methoxycinnamic acid	Inactive
Isatinic acid	0.002
Atrolactic acid	Inactive
Vulpinic acid	Inactive

[1] The curvature is confined to a short region at the top of the coleoptile.

The foregoing compounds tested by Haagen Smit and Went on the Avena coleoptile are also more or less active in the pea test (with the exception of cinnamic acid).

The following compounds were found inactive in the pea test: 2-ethyl-3-indole acetic acid, coumarin, *o*-coumaric acid, cinnamic acid, α-methyl cinnamic acid, α-phenylcinnamic acid, trans-*o*-methoxycinnamic acid, *p*-methoxycinnamic acid, cinnamic-ethyl ester, benzilic acid, phenylglyoxylic acid, benzoic acid, benzalmalonic acid, *r*-mandelic acid, phenylacetamid, diphenylacetic acid, phenylvalerianic acid, *p*-nitrophenylacetic acid, 2-4-dinitrophenylacetic acid, phenylalanin, indole carbonic acid, phenylaminoacetic acid, acetic acid, propionic acid, butyric acid, chloracetic acid, iodacetic acid, malonic acid, maleic acid, adipinic acid, serine, cysteine, isatin, angelic acid, tiglic acid, crotonic acid, acetophenone, tyrosine, eosin.

Coleoptile cylinders 5 mm. in length were placed in solutions of numerous of the foregoing compounds, and the increase in length measured after 12 hours. The same compounds which brought about linear growth of coleoptile cylinders also gave positive results with the pea test, but in numerous instances the same compounds failed to induce curvature in the Avena coleoptile.

Thimann (1935*c*) has recently studied the physiological activity of 3-indole acetic acid (β-indole acetic) and its analogues which contain no nitrogen; these are 3-indene acetic and 1-coumaryl acetic acid. They contain carbon and oxygen atoms, respectively, in place of nitrogen. All three compounds possess the unsaturated five-membered ring, fused to benzene, with the acetic acid side chain. Both analogues produce cell elongation in the Avena coleoptile, are active in root formation, inhibit root

elongation, etc., hence are evidently true growth-promoting substances. 1-Coumaryl acetic acid fails to produce curvature in the Avena coleoptile either because its transport is not polar, that is, it may move in all directions and hence not bring about elongation of one side of the coleoptile, or because it may lack the ability to penetrate the tissues readily. 3-Indene acetic acid is as effective as 3-indole acetic in the Avena test, but it is transported more slowly. The main point brought out by Thimann is that the ability to be transported and the ability to act as a growth substance are separate and distinct properties. A given compound may possess one quality and not the other.

SUMMARY

Numerous sources have been discovered from which growth substances may be prepared in the chemically pure state, and the inclusive term *auxin* has been proposed for chemical usage; it may be employed interchangeably with the physiological terms *growth substance, growth hormone,* etc.

From human urine it is possible to prepare crystalline auxin *a* (auxentriolic acid); and from maize oil, malt, etc., crystalline auxin *b* (auxenolonic acid). The empirical formula of the former is $C_{18}H_{32}O_5$; of the latter, $C_{18}H_{30}O_4$. Still another growth substance, heteroauxin (3-indole acetic acid), may be prepared from urine, yeast, Aspergillus, Rhizopus, etc. It may be produced synthetically also. Its empirical formula is $C_{10}H_9O_2N$.

Auxins *a* and *b* are about equally effective. One milligram of either compound, if unilaterally applied in agar blocks, is capable of bringing about a curvature of 10 degrees in 50,000,000 decapitated coleoptiles. Heteroauxin is approximately one-half as effective.

Crystals of auxin *a* have a melting point of 196; auxin *b*, 183; and heteroauxin, 165°C. Auxins *a* and *b* are stable to heat and light but become physiologically inactive after storage for a few months. Auxin *a* is stable in the presence of acid but is sensitive to alkali, while auxin *b* is sensitive to both. Heteroauxin is sensitive to acid but stable in the presence of alkali.

Numerous chemically diverse synthetic compounds have been found to induce responses in plants similar to those produced by the auxins just described; these other substances are physiologically effective only in much higher concentrations.

CHAPTER IV

THE OCCURRENCE AND FORMATION OF GROWTH SUBSTANCES

The presence and activity of growth substance were demonstrated first in the phototropic and geotropic curvatures of the Avena coleoptile. Later it became clear that growth-regulating substances, that is, hormones, are widely distributed in the plant kingdom. Their presence in any part of an organ or in a culture substratum may be shown in any one of several ways, *e.g.*, by their growth-promoting effect on decapitated Avena coleoptiles. A survey of the distribution of these growth hormones in different organs of higher plants and in different groups of higher plants will be given briefly.

OCCURRENCE

Higher Plants. *Growth Substance in Coleoptiles.*—Paál (1918) showed that a growth substance, influencing phototropic curvature, is present in the tip of the Avena coleoptile. In a typical experiment, a coleoptile was decapitated, and one-half of the cut surface was covered with a foil plate. When the tip was replaced in its original position, contact was made only between half of the tip and basal region of the organ. In a short time, curvatures resulted toward the inserted tin-foil plate. From this experiment it was concluded that growth substance present in the non-illuminated tip moves downward unilaterally and produces a curvature. A similar bend is obtained when the excised tip is replaced unilaterally upon the coleoptile stump. Paál carried out these latter experiments with Coix, and later Neilsen (1924) performed similar tests with Avena.

Söding (1924, 1925, 1929) investigated the occurrence of growth substance in the tip of nonilluminated coleoptiles. It may be mentioned here that the growth rate of a decapitated Avena coleoptile was found to be increased by replacement of the tip.

Using coleoptiles of different species of grasses, Stark and Drechsel (1922) and Stark (1924) showed that phototropic

and geotropic stimuli can be transmitted from the excised and replaced tip into the basal region. Conduction occurred not only when the same tip and stump were recombined but also in interspecific and intergeneric combinations of tip and coleoptile stump. Such experiments were carried out on Avena, Hordeum, Secale, and Triticum.

Zollikofer (1928), investigating the growth-substance content in various species of Panicum, found that the effect upon subsequent growth in such recombinations of coleoptile tips and stumps depended upon the systematic relationship existing between the two parts concerned. On the basis of these experimental results, Stark concluded that growth substances are to a certain extent specific. This conclusion is hardly justified, in view of the many factors that might modify the rates of growth in these cross implantations.

Stark (1921*b*) found that when a ring of coleoptile tissue was placed unilaterally upon a decapitated Avena stump, the resulting curvature was *toward* the side with the ring, *i.e.*, growth was inhibited. This observation was confirmed by Nielsen (1924).

Moissejewa (1928) and Söding (1929) studied the distribution of growth substances in the coleoptiles of Zea and Avena. Both investigators came to the same conclusions, summarized by Söding as follows:

> Hormones present in the coleoptile decrease from tip to base. They are most abundant in the apical millimeter, fairly abundant below this, still present but in smaller amounts in the region 2.5 to 5 mm. below the tip, and absent from the base of the coleoptile. The uppermost millimeter of the tip is the principal region of hormone formation. Varying amounts are present in the lower regions, but whether hormone formation takes place there is not certain.

Went (1928*a*) did not obtain tests for the presence of growth substance in basal parts of the Avena coleoptile below 0.7 mm. from the tip, using the agar-block diffusion technique. The growth of the lower parts of the coleoptile suggests that growth substance must be present there, as pointed out by Söding (1929). Thimann (1934), with the chloroform-extraction method, showed that growth hormone was present in decreasing amounts from the tip to the base of the coleoptile. It was suggested (see also Bonner, 1934*a*) that the substance was present in the lower zones

in a "bound form" and could not diffuse out. Later work by Söding (1935*c*, 1936), using the Cephalaria and the Avena tests, has shown that if several pieces of the basal portion of Avena coleoptiles (6 mm. in length) are placed in succession on an agar block, an amount of growth hormone great enough to give test curvatures diffuses out. He concluded that the growth substance is present in the coleoptile base in the same free form as it is in the tip.

Cholodny (1935*b*), Laibach and Meyer (1935), and Pohl (1935) have shown that growth hormone is present in the endosperm. Pohl's experiments show a decrease in growth in length of the coleoptile by as much as 25 per cent, if the seed coat and aleurone layer are removed or punctured so as to allow the outward diffusion of growth hormone from the endosperm. Appropriate tests showed that wounding was not responsible for the decreased growth. Addition of growth-hormone paste to the wounded seeds increased the elongation of the coleoptile to almost the normal length. When wounded seeds were placed for 12 hours in an electric field with the injured region toward the anode, growth hormone was demonstrable in the fluid surrounding the anode, and the coleoptiles averaged only 30 mm. in length while the controls were 40 mm. Growth hormone added to the seeds that had their supply removed caused normal growth in length of the coleoptiles. It was concluded that growth hormone is not produced in the coleoptile tip but is given off by the endosperm (presumably moved upward in the vascular bundles) and activated by the tip.

When an Avena coleoptile is decapitated, the center from which growth substance is distributed is cut off; hence the growth rate of the stump is reduced for some time. After a few hours, growth is renewed due to a "physiological regeneration" at the upper end of the coleoptile stump. This phenomenon has been investigated by Söding (1925), Dolk (1926), Gorter (1927), Tendeloo (1927), Beyer (1928*a*), Söding (1929), Tsi-Tung Li (1930), etc. Söding (1929) showed that the uppermost millimeter of the "physiologically regenerated" tip is the new center from which growth substance is dispersed. "Regeneration" takes place to the same extent whether 1 to 1.5 mm. or 5 to 6 mm. is removed (see p. 90).

Heyn (1935) collected the growth substance from "physiologically regenerated" Avena coleoptiles into agar blocks.

Determinations of the diffusion coefficient of this "regenerated" growth-promoting substance yielded a mean value of $D_{22} = 0.434$ as compared with the theoretical value of 0.416 for auxin *a*, which indicates that "regenerated" growth substance is probably identical with ordinary auxin.

The investigations of Kögl, Haagen Smit, and Erxleben (1934, Mitt. XII) have led to the identification of auxin as the chemical growth activator in Avena. Further details of this work may be found in Chap. III. That the growth substance found in the Avena seedling is auxin has been substantiated further by Heyn (1935) who found the coefficient of diffusion of the extracted substance approximately the same as that of ordinary auxin.

Foliage Leaves.—Van der Weij (1933*c*) demonstrated the presence of growth substance in young leaves of *Elaeagnus angustifolius*, Thimann and Skoog (1934) in *Vicia Faba*, and Koning (1933) in Ipomoea. With the Avena technique, Avery (1935) obtained quantitative information in regard to the occurrence of a growth substance (probably auxin) in the leaves of Nicotiana. It was found to be plentiful in the young leaves and less abundant in the older ones.

Growth Substance in Hypocotyls, Shoots, and Flower Stalks.—Although the investigations dealing with growth hormones in plant shoots are still few, it is clear, nevertheless, that growth substances are widely distributed in both mono- and dicotyledonous stems.

That the growth of flower stalks is decreased greatly by removal of the inflorescence has been shown by Söding (1926) for *Cardamine pratensis, Cephalaria tatarica, Chrysanthemum leucanthemum, Heliopsis laevis*, and *Helenium autumnale*. A similar observation has been reported by Uyldert (1928) for Bellis. Some genera, for example, Symphoricarpos and Rheum, show a growth substance to be present, although they do not react when the Avena coleoptile is employed as the test object (Söding, 1935*b*). Oosterhuis (1931) showed that stem growth in *Asparagus plumosus* and *A. Sprengeri* is regulated by axillary and terminal buds. These results indicate that growth substances are active in these plants, a point made clearer by the fact that Uyldert (1928) and Nielsen (1930*a*) showed that the flower stalk of Bellis reacts to the application of growth substance. No definite demonstration of the presence of growth substance

in these plants has been made by the Avena test. Using agar blocks, Söding (1932*b*) extracted growth substance from *Heliopsis laevis* and Ornithogalum but was unsuccessful with *Cephalaria tatarica*, as with Symphoricarpos and Rheum mentioned above. It was possible to obtain positive tests in the latter, however, by testing with agar blocks applied to seedlings of the same species (Söding, 1935*b*). Fliry (1932) extracted growth substance from the Helianthus plumule (see also Beyer, 1925). Tests for growth hormones in Tradescantia (Uyldert, 1931) and in beech, oak, and pine (Huber, 1931) gave negative results. Czaja (1934) later showed that growth substance is present in the bursting buds of various woody plants (*Aesculus hippocastanum* and several others).

Van Overbeek (1933) and Dijkman (1934) have published extensive investigations on the occurrence of growth substance in seedlings of Raphanus and Lupinus. In Raphanus it is formed by the cotyledons from which it can be extracted; if the cotyledons are removed, the hypocotyl tip then assumes the function of formation. The situation is somewhat different in Lupinus (Dijkman, 1934) and *Vicia Faba* (van der Laan, 1934). In these plants, there appears to be no center of production; growth substance is present in all growing regions. It is probably distributed more or less equally throughout the Phaseolus seedling (unpublished work of Boysen Jensen). Thimann and Skoog (1934) found that the terminal bud of *Vicia Faba* produces it, as do also the lateral buds during active growth.

Laibach and Meyer (1935) have demonstrated that the amount of growth substance occurring in Helianthus varies according to the stage of development; it is abundant during seed germination and early growth, relatively scarce during the period of vigorous vegetative activity, and high again as the plant comes into fruit (Fig. 19). The tests were made by taking 20 g. of fresh material from different parts of the plant at different stages of development. Growth substance was extracted from this material by boiling in a small amount of acidulated 96 per cent alcohol. The concentrated extract was mixed with lanolin and applied unilaterally to Avena coleoptiles.

The investigations of Schmitz (1933) indicate that all the growing regions of stems in many genera of grasses contain growth substance. Although no growth substance was found in mature

nodes, displacement of the axis from its usual vertical position apparently brought about a renewed production of the growth-promoting hormones.

Boysen Jensen investigated the growth substance of potato tubers, extracting it in alcohol. From his results it seems clear that dormancy in potatoes is not conditioned by a lack of such substances.

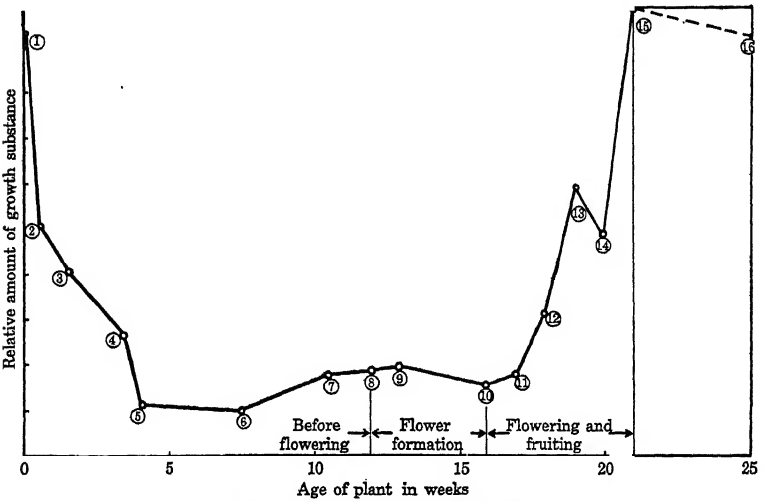


FIG. 19.—Presence of growth substance in *Helianthus* at different periods in the life cycle. The growth hormone is reported to be abundant during seed germination, relatively scarce during the period of vigorous vegetative growth, and high again at the time of seed formation. The height of the plants at the different stages was as follows: 1) seeds, 2) 2 cm., 3) 10 cm., 4) 25–35 cm., 5) 40 cm., 6) 70 cm., 7) 150–180 cm., 8) to 16) about 200 cm. (unpub. data). (*After Laibach and Meyer*, 1935.)

Roots.—A great many investigations on the occurrence of growth substance in root tips have been carried out without much success. Cholodny (1933*b*) showed that *Zea mays* root tips produced a growth effect about equal to that of Avena coleoptile tips. Growth substance can be extracted from root tips of *Zea curagua* and *Vicia Faba* when agar containing 10 per cent dextrose is used instead of pure agar (Boysen Jensen, 1933*b*). This subject is treated at greater length elsewhere (see p. 25).

Pollen.—According to the investigations of Laibach (1932, 1933*a*), Maschmann and Laibach (1932), and Laibach and Maschmann (1933), the pollen hormone, which Fitting (1909)

first demonstrated in orchid pollinia, is probably identical with the growth substance of coleoptile tips. The pollen of Hibiscus also promotes growth in Avena (Laibach, 1932). Sequoia pollen has been found even more active than the pollen of orchids (Thimann, 1934).

Fruits and Seeds.—Growth substance has been demonstrated in peas, beans, lentils, tomatoes, oranges, and lemons (Maschmann and Laibach, 1933). Kögl, Erxleben, and Haagen Smit (1934, Mitt. IX) isolated auxin *a* and auxin *b* from oil made from maize embryos and from barley malt. Cholodny (1935*b*) has reported the presence of a growth-promoting substance in the hydrated endosperm of sprouting oats and corn seeds which acts in the same way as the growth hormone present in the coleoptile. Using the lanolin-paste method, Laibach and Meyer (1935) found that acid-alcohol extracts of both killed and germinating seeds of Avena and Helianthus contained a substance that stimulated growth of the Avena coleoptile; it was present in greatest concentration during early germination. It was present in high concentration again at the time of fruit formation. The embryo of Triticum also contains abundant growth substance (Thimann, 1934).

Lower Plants.—The presence of a growth substance was first demonstrated in lower plants by Nielsen (1930*b*) when he showed that two fungi, *Rhizopus suinus* and *Absidia ramosa*, produce it when subjected to proper culture conditions. A series of investigations concerning the nature of this fungus substance has led to the conclusion that it is probably heteroauxin (Thimann, 1935*b*). The production of growth substance occurs generally among bacteria (Boysen Jensen, 1931*a*). Besides two bacteria isolated from saliva, the following are productive: *Bac. mycoides*, *Bac. subtilis*, *Bact. xylinum*, *Bact. radiobacter*, *Bact. dentrificans*, *Bact. coelicol.*, *Bact. coli*, *Bact. vulgatus*, *Mycobact. album*, *Mycobact. lacticola*, and *Proteus vulgaris*. Sixteen of 20 investigated bacteria formed growth substance.

Besides those already mentioned, various other fungi are growth-substance formers. Particularly large amounts are produced by *Aspergillus niger* (Boysen Jensen, 1931*b*). It has been demonstrated also in baker's yeast (Nielsen, 1931*b*), *Rhizopus Delemar*, *R. nigricans*, *R. tritici*, and *R. reflexus* (Kögl and Haagen Smit, 1931, Mitt. I), *Boletus edulis* (Nielsen, 1932), and Penicillium (Guttenberg, 1933). In further studies, Kögl and

Kostermans (1934, Mitt. XIII) have identified heteroauxin in yeast, *Rhizopus nigricans*, and *Aspergillus niger*.

Van der Weij (1933*a*, *b*) demonstrated growth substance in an alga *Valonia macrophysa*. None was found in the setae of *Pellia epiphylla* (Overbeck, 1934).

Plant Products.—In the light of the wide distribution of growth substance in higher and lower plants, it is reasonable to expect its presence in some of the plant products of commerce. Seubert (1925) found growth-promoting properties in malt. Kögl, Haagen Smit, and Erxleben (1933, Mitt. VII) reported a significant amount of it in salad oil (perhaps peanut oil); however, the growth substance was not present in a free form. It could be demonstrated after saponification by treatment with hydrochloric acid or pancreas lipase. Not all salad oils that were tested gave positive results; in certain other oils, for example, maize oil, free growth substance was found. Maschmann and Laibach (1933) demonstrated growth substance in flour made from oats and rye and found it in various kinds of bread. According to Kögl, Erxleben and Haagen Smit, (1934, Mitt. IX), both auxin *a* and auxin *b* occur in peanut, sunflower, mustard, and linseed oils.

Boysen Jensen (1931*b*) obtained a significant test for the growth hormone in a preparation of Witte peptone. The hormone may have been formed by the action of bacteria or fungi during the manufacture of the product.

Human and Animal Organisms.—Seubert (1925) made the remarkable discovery that significant amounts of growth substance are present in human saliva; this was the first time that the compound was found outside plants. Kögl and Haagen Smit (1931, Mitt. I) (see also Kögl, 1932, and 1932, Mitt. II) showed later that human and animal urine contains a great deal of growth substance.

The presence of growth substance in animal organs has been demonstrated by Maschmann (1932), Maschmann and Laibach (1932, 1933), and Kögl, Haagen Smit, and Tönnis (1933, Mitt. VIII) and in malignant growths by Maschmann (1932). Whether or not it plays a part in tumor growth is doubtful (Kögl, Haagen Smit, and Tönnis, 1933, Mitt. VIII), even though carcinoma showed a higher concentration of growth substance than neighboring normal tissue. Navez and Kropp (1934)

obtained extracts from the eyestalks of the crustacean Palaemonetes which promoted growth of the Avena coleoptile. Growth substance is often present in follicle-hormone preparations, but the crystallized follicle hormone exerts no effect resembling that of the plant-growth substance (Kögl and Haagen Smit, 1931, Mitt. I; Harder and Störmer, 1934).

THE FORMATION OF GROWTH SUBSTANCES

Lower Plants.—The conditions that influence the formation of growth substances by fungi have been investigated in considerable detail. Nielsen (1930*a*, *b*) found that *Rhizopus suinus* forms growth substance in the presence of glucose-ammonium tartrate solutions, when cultured on a more or less solid substratum. However, *Aspergillus niger* does not form growth substances in glucose-nitrate or glucose-ammonium solutions; relatively large amounts are formed in glucose-peptone solutions on a fluid substratum. Boysen Jensen (1932) studied a series of compounds from the point of view of their possible use in the formation of growth substance. Investigations on a series of amino acids yielded the following results: Tyrosin was found to be an excellent growth-substance former; the same is true for two cyclic amino acids, phenylalanin and histidin, and two aliphatic amino acids, leucin and lysin. Glycocoll, alanin, arginin, asparagin, cystin, and prolin were found to be ineffective. The amount of growth substance produced is increased greatly with temperature, ten times as much being formed at 36 to 37°C. as at 22 to 24°C. (*cf.* Sakamura and Yanagihara, 1932).

Thimann and Dolk (1933) (see Bonner, 1932) found that growth-substance production by Rhizopus growing on a peptone-dextrose culture medium is greatly increased by aeration; the increase is proportional, within limits, to the extent of the aeration. The optimum yield of growth hormone is reached after 10 days at a temperature of 35 to 36°C. Its production takes place during the quiescent stage after rapid vegetative growth has ceased. Witte peptone, prepared from fibrin, was superior to Merck's Fleischpeptone, a fact shown later (Thimann, 1935*b*) to be due to the presence of tryptophane in the Witte peptone; the growth substance was identified as heteroauxin.

The yield of heteroauxin proportionate to the aeration of the cultures (Thimann, 1935*b*) has been explained by the use of

oxygen in the typical oxidative deamination of the tryptophane. Other amino acids which yield significant amounts of the active growth substance in mold cultures can also be converted readily into 3-indole acetic acid.

Higher Plants.—Very little is known about the conditions required for the formation of growth substances in higher plants. In many plants the process is not influenced by unilateral light or gravity (Chaps. VIII and IX). According to van Overbeek (1933), light has an effect on its formation in the cotyledons of Raphanus. The very young cotyledons of etiolated and of illuminated plants contain the same amounts of growth substance, probably derived from storage in the seed; however, seedling plants grown in darkness lose their ability to form growth substance rather soon, while normal plants placed in the light retain this ability for a long time. Navez (1933*c*) found that illuminated *Lupinus albus* seedlings contain twice as much growth substance in the apical regions as darkened plants. It seems probable from the observations of Cholodny (1935*a*, *b*), Laibach and Meyer (1935), and Kögl, Haagen Smit, and Erxleben (1933, Mitt. VII), that the growth substance stored in seeds is liberated in an active form following hydrolysis and the activity of enzymes. Pohl (1935) has concluded that the growth hormone in Avena is moved from the endosperm up to the coleoptile tip where it is activated before being moved downward.

Avery (1935) found that a growth hormone was produced in the young growing leaves of tobacco in the light; it disappeared slowly when plants were placed in the dark. Chesley (1935) observed that wheat seedlings sprouted in the light were less sensitive to X radiation (which, according to Skoog, 1935, destroys auxin) than those germinated in darkness. This observation may be explained on the supposition that a greater concentration of auxin was present in the illuminated plants; hence greater doses of X rays were required to inhibit growth. Went (1935*b*) has stated that root-forming substances are produced in leaves growing in the light, the red and orange wave lengths being especially effective. Küstner (1931) observed that the activity of growth substance obtained from urine was increased by red light and decreased by shorter wave lengths. Went (1928*a*) found a decrease of 18 per cent in the amount of growth substance given off by the Avena coleoptile when illuminated with 1,000

meter-candle seconds as compared with plants kept in darkness. Although these various observations disagree in certain points, they may be brought into harmony later by further investigations on the problem under controlled conditions.

Mature grass nodes which contain no growth substance have been reported to regain their ability to form it when stimulated by gravity (Schmitz, 1933). This interesting phenomenon has not yet been explained satisfactorily.

That the formation of growth substance is influenced by temperature has been shown by the experiments of Hawker (1933). Root tips of *Lathyrus odoratus* grown at 20°C. produced more of the hormone controlling root growth than similar tips grown at 5°C. (Fig. 62). The influence of temperature has not yet been studied carefully enough to permit any statement with respect to a "temperature coefficient" for the process.

As a result of certain toxic effects, the ability to form growth substance may be diminished or entirely lost. Roots of *Vicia Faba* and *Pisum sativum* that have been treated with erythrosin react ageotropically (see Boas and Merkenschlager, 1925) and contain much less growth substance than normal roots (Boysen Jensen, 1934). Van der Laan (1934) showed that ethylene decreases the production of growth substance in Avena and in *Vicia Faba* but not its utilization, for, when growth substance is directly applied, growth is not affected.

Studies by Skoog (1935) have shown that X rays inactivate both auxin *a* and 3-indole acetic acid in aqueous solutions. The growth hormone normally present in plants is partially inactivated also by moderate treatments. The formation of auxin in green plants (Pisum and Vicia) is inhibited by irradiations of 300 to 1200 R. When Avena coleoptiles were irradiated with dosages of the same magnitude, no effect upon transport or formation of growth hormone could be found. The treated coleoptiles grew only slightly less than the controls. Applications of growth hormone to the irradiated coleoptiles showed that the capacity for growth was not affected by the X-ray treatment. Failure of irradiation to alter the growth rate markedly in Avena may find its explanation in the recent observation of Pohl (1935) that the growth hormone is not formed in the coleoptile but moves from the endosperm up to the coleoptile tip, where it is activated and distributed downward.

Hence, formation of the hormone probably would not be influenced by irradiation of the coleoptile alone.

Production of Auxin in Human Urine.—Kögl, Haagen Smit, and Erxleben (1933, Mitt. IV) prepared a crystallized growth substance from human urine. It was obtained from the bicarbonate fraction in the preparation of the follicle hormone from the urine of a pregnant woman. Later it was prepared from mixed urine from the Utrecht clinics. The total growth-substance content of normal urine is about 80 per cent auxentriolic acid (auxin *a*) and 20 per cent 3-indole acetic acid (heteroauxin) according to later investigations by these same workers (Kögl, Haagen Smit, and Erxleben, 1934, Mitt. XI and XII).

Human urine ordinarily contains from 1 to 2 mg. of growth substance per liter, irrespective of age, sex, or such pathological conditions as carcinoma and tuberculosis (Kögl, Haagen Smit, and Erxleben, 1933, Mitt. VII). Auxin elimination was somewhat higher than normal in cases of diabetes, probably owing to an abundance of fats. In the average individual the auxin excretion comprises about 20 per cent of the quantity ingested. The excretion in the first few hours after a meal has the highest content of auxin (Fig. 20*A*). Diets high in fats are more effective than others in producing an "auxin peak" after meals (Fig. 20*B*); the ingestion of hydrogenated fats is without effect. If arachis (peanut) oil is subjected to the action of lipase, its products possess growth-promoting properties; this may explain why diets high in fats lead to high auxin production in the urine.

The source of growth substance in the saliva and urine of the human body still remains to be explained satisfactorily (Kögl, Haagen Smit, and Erxleben, 1933, Mitt. VII). A portion of the growth substance in saliva doubtless is produced by bacteria in the mouth. That it is produced probably by bacteria in the intestines is indicated by the fact that human feces carry a significant amount of growth substance (Kögl and Haagen Smit, 1931, Mitt. I); this is probably largely absorbed by the body. According to Kögl and his coworkers, the growth substance produced by intestinal bacteria constitutes only a small portion of the auxin in urine. Significant amounts of growth substance are consumed in food. The hourly elimination of auxin in urine shows a maximum at eight o'clock in the evening following the main meal at six o'clock. The ingestion of grape sugar, starch,

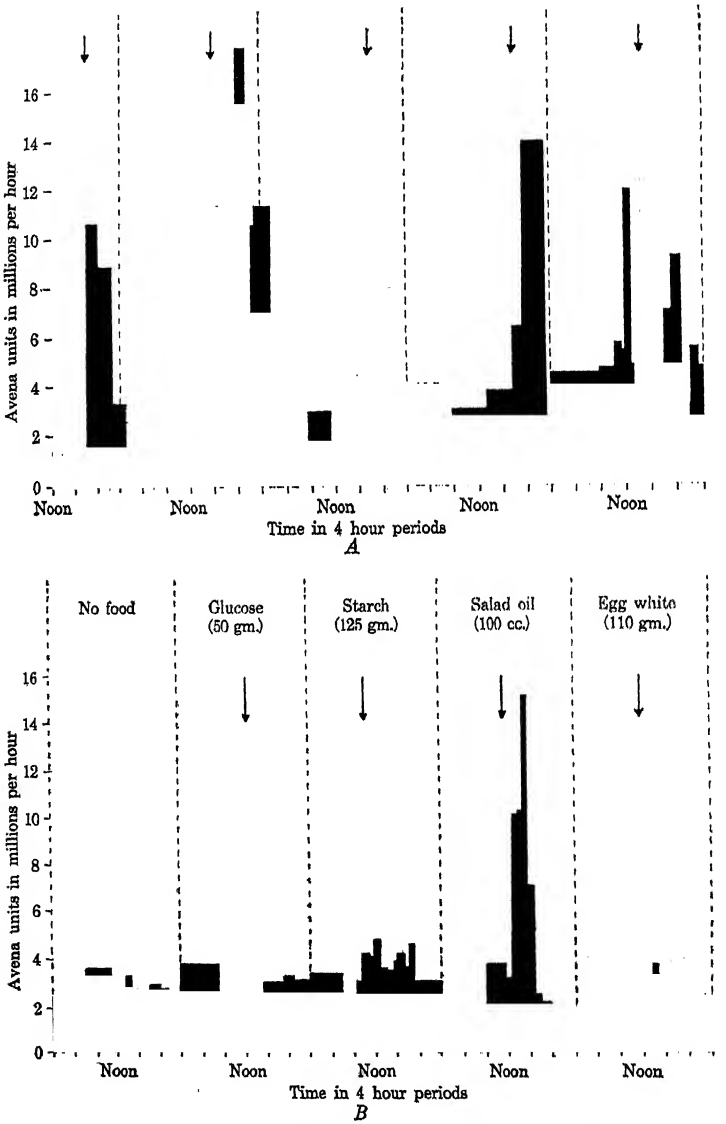


FIG. 20.—Auxin content of human urine. *A*, hourly elimination of auxin in urine increases markedly following a meal, indicated by an arrow. *B*, auxin elimination after ingestion of various foodstuffs shows a sharp rise in the case of salad oil, which probably contains some crude form of the growth hormone. One hundred cubic centimeters water were consumed hourly. (*After Kögl*, 1933*b*.)

and white of egg does not cause increased growth-substance production, but salad oil brings about a decided increase. It would be of interest to investigate auxin content of urine after consumption of Witte peptone, since this substance is an excellent growth-substance former in fungi. Kögl and his coworkers assume that 0.1 to 1.0 mg. of auxin is consumed daily in foods, a fact that would explain in part the normal daily elimination of auxin, amounting to 2.0 mg. However, three people showed a daily elimination of 8 to 10 mg. of auxin (Kögl, Haagen Smit, and Erxleben, 1934, Mitt. XI), of which a higher percentage than normal was 3-indole acetic acid. Since this large amount could hardly be supplied by the food consumed, one is led to conclude that the human body must be capable of forming auxin, probably by decomposing or recombining substances supplied with the food consumed.

SUMMARY

On the basis of the Avena-coleoptile test, hormones have been demonstrated in many kinds of plants belonging to widely separated taxonomic groups. They have been found in the following higher plants: coleoptiles of Avena, Hordeum, Secale, Triticum, and Panicum; foliage leaves of Nicotiana, Eleagnus, Vicia, Ipomoea, etc.; hypocotyls and stems of Raphanus, Lupinus, Vicia, and many others; winter buds of numerous species that are coming out of their dormant period; roots of Zea and Vicia; pollen of orchids, Hibiscus, and Sequoia; fruits and seeds of peas, beans, tomatoes, oranges, lemons, maize, barley, oats, sunflowers, etc. Growth hormone has been found in the majority of the lower plants that have been tested: among the molds, Rhizopus, Absidia, Aspergillus, Penicillium; in the bacteria, Bacillus, Bacterium, Mycobacterium, Proteus; the fleshy fungus, Boletus; and the alga, Valonia. No doubt, further investigations will reveal the occurrence of growth-regulating substances in all plants.

The presence of growth hormones in the commercial products of plants has been investigated, and among the materials that have yielded growth substance are malt, flour, and the oils derived from maize, peanut, sunflower, mustard, and flax.

The richest source of plant-growth substances (auxin *a* and heteroauxin) has been found in human urine. They have been

demonstrated also in higher animal tissues, both normal and pathological, in saliva and in crustaceans, etc.

The question of growth-substance formation is of very great importance. There is some indication that the plant-growth hormones found in animals may have their origin in the materials supplied in plant foods. The action of the enzyme lipase upon peanut oil is known to result in the production of growth hormone. Diets rich in fats increase the auxin content of urine.

The formation of growth substances in molds and bacteria has been studied in relation to the character of the nutrient substratum; the following substances in the medium were favorable for growth-substance formation: glucose peptone, glucose-ammonium tartrate, and tryptophane, tyrosin and several other amino acids.

It has been learned recently that the growth substances stored in seeds of higher plants are of great significance in germination and seedling growth. The amount of growth substance in seeds is increased upon hydration of the stored foods during germination. Light seems to promote its formation in seedlings and in the growing parts of young leaves, perhaps also in meristems. Localized chemical changes in plants, *e.g.*, increased acidity, may release active growth hormone from the inactive salt form. Ethylene treatment apparently decreases the production of growth substance in Vicia and Avena. Within limits, higher temperatures have been found to result in increased production of the substances both in lower plants (molds) and in the roots of higher plants. In view of the limited information existing at present on this important subject, an extremely promising field is open for further investigation.

CHAPTER V

THE TRANSPORT OF GROWTH SUBSTANCES

The growth substances that are formed in different parts of a plant are transported to other regions where they exert specific physiological effects on the tissues. For example, the substance dispersed from the coleoptile tip influences the enlargement of cells in tissues at some distance away. Following the definition of Starling (1914), such a substance is a hormone, and the problem that confronts us now is to explain the mechanism by which the plant-growth hormones are translocated from the place of their formation (or center of distribution) to the region of their functional activity.

The Avena Coleoptile.—The great majority of investigators interested in the transport of plant hormones have used the oat coleoptile as an experimental object. It has been shown that the substance controlling growth in young Avena seedlings is distributed from the extreme apical portion of the first leaf above the cotyledon (coleoptile).

The Conducting Tissues.—From the facts presented in the earlier chapters, it is clear that there is abundant movement of growth substance from the tip toward the base of the coleoptile. Under normal conditions, migration of the substance takes place almost exclusively in a longitudinal direction, and very little is transported transversely. In naturally or artificially twisted coleoptiles, conduction of a stimulus (*i.e.*, the transport of growth substance) ordinarily follows the course of the vascular bundles (Tammes, 1931).

Rothert (1894) investigated the conduction of the phototropic stimulus and thus the transport of growth substance in the Avena coleoptile. Since it was found that conduction was not hindered when both vascular bundles were severed, it was concluded that the stimulus is transmitted in the parenchyma. Although there may have been sources of error in Rothert's experimental method, his conclusion that growth substance can move in the paren-

chymatous tissue has been corroborated (Went, 1928*a*; Laibach and Kornmann, 1933*b*).

Polarity of Transport.—The movement of growth substance in the Avena coleoptile has been found to be a polar phenomenon, occurring only in a basipetal and not in an acropetal direction. Beyer (1928*a*) showed that the transport of growth substance from a decapitated and unilaterally replaced tip into the coleoptile stump was inhibited if a ring segment of coleoptile was inverted and placed between the tip and the base. If the inserted coleoptile ring was oriented normally, however, the growth substance migrated downward freely. Went (1928*a*) reached a similar conclusion in regard to the polar nature of movement: agar blocks were placed on the two terminal cut surfaces of coleoptile cylinders, 2 mm. in length (Fig. 2) (Went). The upper block contained growth substance, and the lower one was plain agar. After some time, both blocks were analyzed for growth substance in order to determine the amount of transport. Very little growth substance was present in the lower block when the coleoptile cylinders were in an inverted position, showing that growth substance did not migrate from an agar block placed at the morphological base to a block placed at the morphological apex. Van der Weij (1932), using a similar method, found a significant amount of transport in inverted coleoptile cylinders 1 mm. long, but this was thought to be due to diffusion of the growth substance in the water adhering to the coleoptile. In agreement with other investigators, the general conclusion was reached that probably movement of growth substance can take place only basipetally through living tissues. It was found, in addition, that growth substance can be transported against a gradient, *i.e.*, from a place with a lower concentration to another with a higher concentration. If an agar block placed at the morphologically basal end contained more growth substance than a block placed at the apical end, the concentration was soon diminished in the upper block, although an increase in growth substance in the lower block could not be shown. A concentration at the basal end of a coleoptile cylinder ten to twenty times that at the apical end does not impede basipetal transport. Gravity appears to have only a very slight effect upon the direction of movement, which takes place just as well upward as downward in a morphologically basipetal direction.

Rate and Amount of Transport.—Van der Weij (1932, 1934) also carried out experiments dealing with the rate and the amount of growth-substance transport and with the effect of various external factors upon the magnitude of these phenomena. The experimental technique was essentially like that of Went (1928*a*). Coleoptile cylinders provided at the upper end with agar blocks containing growth substance were placed erect upon agar that contained none. Increase of concentration in the lower block was investigated over a period of time. The original concentration in the upper blocks was measured by the angle of deviation that they could produce when placed unilaterally on decapitated coleoptiles; the concentration found in the lower blocks was given in percentage of the original. Since the results of these experiments were usually portrayed graphically, a complete estimate of the experiments is not always possible. According to van der Weij, the capacity of transport may be considered as the amount of growth substance found per unit of time in the lower agar block after equilibrium has been reached. This value was determined from the curves that were produced when the increase of growth-substance concentration in the lower block was plotted as a function of the time. The rate of transport is the distance through which the growth substance moves per unit of time. In order to determine this value, the exact moment at which the growth substance starts to enter the lower block must be ascertained. The period of time required for the substance to traverse the known length of the coleoptile and first appear in the lower block was determined from the point of intersection of the concentration curves on the time axis.

Close inspection of van der Weij's curves gives the impression that some of the extrapolations are arbitrary. For example, it would be possible to conclude that the rate of transport decreases with an increase in the temperature; but, on the other hand, from experiment 126 (1932), it might be concluded that it increases with rising temperature. The points of the curve fluctuate so much that the point of origin of the curve cannot be determined with certainty. Hence it is not definitely established that the rate of growth-substance transport is independent of the temperature.

Rate of Transport.—The conclusions that may be drawn from van der Weij's experiments are significant, nevertheless.

The rate of transport, about 10 mm. per hour, is much greater than can be accounted for by diffusion. It is not influenced by the length of the tissue through which it is moved, is very little affected by temperature, and is practically independent of the concentration gradient.

Amount of Transport.—In contrast to the rate of growth-substance movement, the capacity of transport, (*i.e.*, the amount transported per unit of time, sometimes called intensity of transport) is inversely proportional to the length of the coleoptile at 0°C., but at higher temperatures it appears to be independent of the length of the portion of the organ in question. If the original concentration of growth substance is increased, the absolute amount does not increase in proportion to the concentration rise, and the "relative" amount of transport consequently decreases. The effect of rising temperature on amount of transport shows an increase up to an optimum at 30 to 40°C. Thimann (1935*a*) has likened the movement of growth substance to the transport of objects along a moving band; the band travels at a constant speed so that the number of objects arriving at the end per unit of time is independent of the length; if not removed from the end, there is an accumulation (transport against the gradient) (see also van der Weij, 1932).

X Irradiation and Transport.—Recent experiments by Skoog (1935) have shown that the movement of growth hormone in the Avena coleoptile is not affected by moderate dosages of X irradiation (30 Röntgen units per minute at 900 kv. and 3 to 4 milliamperes for 200 minutes and more).

Anaesthesia and Transport.—In a later paper, van der Weij (1934) has shown that anaesthetizing with ether abolishes polar movement and stops all transport beyond that which can be accounted for by diffusion. This anaesthetic inhibition is reversible within certain limits of ether concentration. Small concentrations of the anaesthetic had no effect upon the normal rate of the transport, but increasing concentrations of ether decreased the amount of transport. The nonpolarized movement of growth substance under conditions of complete narcosis was attributed to diffusion in adhering water films outside the living cell material. Van der Laan (1934) reported that ethylene (0.005 to 0.0005 per cent of gas) had no effect upon the transport but inhibited the formation of growth substance in

Avena. More recent studies (Crocker, Hitchcock, and Zimmerman, 1935) suggest that ethylene itself may be a plant hormone, though the experiments of Michener (1935) have failed to show any growth-promoting effect of ethylene applied in a series of concentrations to Avena coleoptiles.

Growth-substance Transport in Shoots and Roots.—Studies on the translocation of growth substance in the stems of seedlings and mature plants have brought some new facts to light in recent years.

Van Overbeek (1933) observed transport of growth substance in the hypocotyls of *Raphanus sativus*. He found that movement was basipetal and was not influenced by general illumination. The work of Thimann and Skoog (1934) would suggest that auxin can travel from the terminal bud downward in the stem where it may inhibit lateral bud development. Furthermore, Snow (1929*a*) found that the downward movement of this bud-inhibiting substance takes place chiefly in living cells. The production of growth-promoting substance in the young leaves of Nicotiana and its accumulation and polar movement (in a basipetal direction) in the veins (Avery, 1935) is of considerable interest from the standpoint of the role of growth hormones in normal plant growth.

Studies on the direction of transport in *Coleus hybridus* (Mai, 1934) gave the following results: in young, still growing petioles, movement took place only in a basipetal direction; in fully grown petioles, movement occurred in either direction; and in old and falling petioles, there was little or no movement. In stems of Coleus, Vicia, and Phaseolus, and in hypocotyls of Vicia, Phaseolus, and Lupinus, Mai found that (in all but one instance) transport occurred in a basipetal direction only.

Experiments carried out recently at the Boyce Thompson Institute have shown that growth substances may be taken up from the soil through the roots (Hitchcock and Zimmerman, 1935). Following their entry and upward passage into different parts of the plant, their effects upon growth, root formation, etc., are very striking. These observations appear to contradict previous conclusions as to the polar movement of growth substances. Inasmuch as the upward migration was greater than 47 cm. per hour under optimum conditions, it is probable that translocation of the substances in this instance was in the trans-

piration stream. The *dead* cells of the xylem are doubtless the pathways of transport; hence this observation does not contradict the evidence for polar movement through *living* tissues. Characteristic bending, proliferation, and rooting responses result from the upward movement of growth substances through stems (Fig. 41). Absorption and movement were delayed or prevented under conditions of low transpiration. Movement was observed in both directions through dead tissue.

The Mechanism of Growth-substance Transport.—Any attempt to formulate an explanation of the mechanism of growth-substance movement should explain the rate as well as the polarity of transport in agreement with the results reported for the Avena coleoptile. A satisfactory explanation must be in agreement with the transport phenomena observed in other plant organs. Several possibilities will be examined.

Diffusion.—That all growth-substance movement cannot be by diffusion is clear from the magnitude of the rate of transport. Went (1928*a*) has computed that two hundred times more growth substance is moved in a given time than could be accounted for by diffusion. Then, too, the conclusions of van der Weij concerning the amount of transport at higher temperatures are not compatible with the conception of movement as a process of diffusion. If diffusion were concerned, the amount moved would have to be inversely proportional to the length of the coleoptile cylinder and directly proportional to the concentration of the original solution. Experimental evidence has shown that this is not the case in the Avena coleoptile.

The theories offered in explanation of the translocation of nutritive materials in plants do not suffice for movement of growth substance in the Avena coleoptile. The movement of such materials is not polar and is bound up with certain conducting cells which may not enter into the question of growth-substance transport. Certainly too little is known yet to make a final statement regarding the role of diffusion in the movement of growth substance.

Protoplasmic Streaming.—Since the time of de Vries (1885) it has been accepted generally that in many cases the movement of dissolved substances is facilitated by protoplasmic streaming. It is possible, therefore, that streaming plays a part in the transportation of growth substance. Brauner (1922) suspected that

his "growth-retarding substance" was moved by protoplasmic streaming. A rapid streaming of the protoplasm in the cells of the coleoptile has been observed by Brauner (1922), Perry (1932), Bottelier (1934), and others. According to Went (1928*a*), the rate of this streaming is of the right order of magnitude to serve as an explanation for the rate of growth-substance transport.

The longitudinal movement of growth substance in Avena apparently is not influenced directly by light (Boysen Jensen, 1933*a*). However, the effect of light upon protoplasmic streaming, the lateral displacement of growth substance, and tropic curvature in Avena is well-known (Bottelier, 1933, 1934; Went, 1928*a*; Boysen Jensen, 1933*a*). Bottelier (1934) found that the retarding action of different wave lengths of light upon protoplasmic streaming paralleled the phototropic effectiveness. Furthermore, the supply of oxygen also has a marked influence upon streaming (Bottelier, 1935).

Van der Weij (1932) questioned the significance of protoplasmic streaming for transport of growth substance because, if protoplasmic streaming plays an essential part, the rate of movement should be dependent upon the rate of protoplasmic streaming and therefore upon the temperature. Since it was found that the rate of transport is not changed by an increase in temperature, van der Weij concluded that growth substance is not moved in the streaming protoplasm but rather in the "resting" plasma membrane, which was considered as "cell-wall substance *in statu nascendi*."

It may be premature to draw far-reaching conclusions from van der Weij's investigations, but it would appear that the transportation of growth substance is connected in some way with living processes. Bottelier (1934) has found that in young coleoptiles of Avena the rate of protoplasmic streaming between 17 and 35°C. is influenced very little by the temperature. However, the intensity of streaming (*i.e.*, the amount of protoplasm in actual rotation) increases with temperature, 30°C. being optimum (Bottelier, 1934). F. A. F. C. Went (1935) has pointed out the existence of similar values for the Q_{10} of protoplasmic streaming (Bottelier) and growth-substance transport (van der Weij) between 16.5 and 24°C. Since the intracellular movement of the hormone by protoplasmic streaming would be considerably more rapid than its observed transport velocity in plant organs, the conclusion seems to follow that cell-to-cell transport must

be the controlling link in the process. The possibility still exists that in some instances protoplasmic streaming may aid in the translocation of growth substances; however, it must be said that even though protoplasmic streaming might aid in explaining the rate, it could not account for the polarity of the movement. This may be governed by circumstances associated with the migration of the substance from one cell to another.

Surface Tension.—For the explanation of the transport of plastic substances in the sieve tubes, van den Honert (1932) directed attention to the possible significance of the active surface force by which certain fluids can move upon the contact surfaces of phases. It has been found, indeed, that auxin spreads itself in a monomolecular layer, and Kögl (1933, Mitt. III) has considered the possibility that auxin may be transported by spreading out in a lipoid layer of protoplasm. Nothing definite can be said at present as to the significance of these hypotheses, except that such a mechanism would be more than ample to account for the velocity and amount of movement.

Electrophoresis.—The significance of electrical potential differences has been considered in accounting for growth-substance transport. Since growth substance is an acid, it might be expected to move toward positively charged regions. Recently Koch (1934), by noting its migration in a polarized agar block, has demonstrated that this is true. Kögl (1933*b*; 1933, Mitt. VI) actually has shown the influence of applied electrical potentials upon the longitudinal transport of auxin toward the positive pole in the Avena coleoptile. It is known that differences in electrical potential are produced in plant organs as a result of the action of unilateral light and gravity (Bose, 1928; Ramshorn, 1934; Waller, 1929; Brauner, 1935), and to them the transverse movement of growth substance in phototropic and in geotropic curvatures has been ascribed. Various experiments have shown that growth curvatures can be produced by potential differences in accordance with predictions on an electrical basis, and it seems probable that electrical potentials influence the transverse transport of growth substance in plants (Koch, 1934). These investigations are treated in more detail in the chapters on photo- and geotropism.

Went (1932) published a somewhat theoretical paper on this question of electrical transport and showed that the direction of

movement of dyes depends upon the charge of the dye and the potential gradient in the plant. It was concluded that growth substance is transported from the aerial portion of a plant toward the root.

The fact that growth substance may be transported both upward (transpiration stream) as well as downward in the axes of tomato, tobacco, artichoke, and other plants (Zimmerman and Wilcoxon, 1935; Zimmerman and Hitchcock, 1935) sheds no particular light on the question of electrophoresis in living tissues. Ramshorn (1934) pointed out that variations in growth brought about by modification of the supply of growth substance might be accounted for by changes in electrical potential. It is difficult to say in all cases whether the observed changes in electrical polarity are the cause or the result of the distribution of growth substance (Czaja, 1935*a*). In view of the few and diverse reports on the subject, it is not possible at present to present a complete explanation of the longitudinal transport of growth substance.

Movement of Growth Substance from Agar Blocks into Decapitated Avena Coleoptiles.—The manner of entry of growth substance into plants from the outside has been studied by Thimann and Bonner (1932). By applying an agar block containing growth substance to each of a series of Avena test plants (for a standard time) and measuring the resulting curvatures, it was found that a constant fraction (13 to 21 per cent) of the growth substance present in the block passes into a given coleoptile. It was calculated that the rate of growth-substance movement from the block into the plant is proportional to the concentration of the substance in the block.

SUMMARY

It is difficult to explain the transport of plant-growth hormones in the higher plants from the place where they are formed to the place where they become functionally active. Movement apparently takes place either through the nonliving xylem cells in the vascular bundles or through the living cells of the phloem and parenchymatous tissues. Translocation through young, living tissues is polar. The substances move in a morphologically basipetal direction, for example, prevailingly from the tip region downward in the Avena coleoptile and from the embryonic

regions downward in the stems and hypocotyls of dicotyledonous seedlings. This polar transport may be reversibly abolished by anaesthesia.

It has been discovered recently that 3-indole acetic acid and other growth substances when added to the soil can be absorbed by the roots and carried upward (apparently in the transpiration stream) into all parts of the plant. This upward movement through dead xylem cells does not necessarily contradict the facts embodied in the foregoing statement concerning prevailingly downward movement through living tissues.

The characteristic migration of growth substance through excised portions of the Avena coleoptile has been studied by applying it in an agar block at one end of the coleoptile cylinder and recovering it in an agar block at the other end. From experiments of this type it has been possible to distinguish two important characteristics of hormone movement, *viz.*, velocity and amount of transport. The rate of movement (velocity) is about 10 mm. per hour, which is considerably more rapid than could be accounted for by diffusion; it is not affected by temperature and apparently is independent of the concentration gradient. The amount moved in unit time (capacity, or intensity of transport) is inversely proportional to the length of the organ at 0°C. At higher temperatures the amount of movement seems to be independent of the length of the pathway. The amount of substance transported is increased by raising the temperature to an optimum in the region of 30 to 40°C.

Attempts to explain the mechanism of transport have been based upon diffusion, protoplasmic streaming, interfacial tension, and electrophoresis. Of these, the last seems to have much in its favor. The acid character of the auxins would suggest that their active radical might be moved toward the positively charged pole in an electrical circuit. This prediction has been verified experimentally in agar as well as in plant tissues under conditions of controlled electrical potential. It is difficult to conclude whether in all cases the observed changes in electrical polarity are the cause or the result of the presence of growth substance. The recent discovery of movement upward in the transpiration stream makes it impossible, at present, to account for all the phenomena of hormone transport on the basis of a single comprehensive mechanism.

CHAPTER VI

THE SIGNIFICANCE OF GROWTH SUBSTANCES FOR THE NORMAL GROWTH OF PLANTS

GENERAL SURVEY OF THE EFFECT OF GROWTH SUBSTANCES UPON GROWTH

Up to the present time, plant hormones have been studied more extensively in relation to cell enlargement than in relation to cell division. Consequently, the following discussion will be confined almost entirely to those problems which deal with the enlargement of cells. In general, it can be said that growth takes place only when growth substances are present. Their distribution and activity have been determined for diverse organs in many kinds of plants; of these the Avena coleoptile is probably the best known.

The Avena Coleoptile. *Structure.*—The roots appear first in the germination of the Avena seed; shortly thereafter the coleoptile appears. The latter is the first leaf above the cotyledon and differs from later leaves in having no lamina. It is a cylindrical sheathing leaf which encloses the embryonic foliage leaves, and its vascular relationship with the cotyledon is somewhat more intimate than that of the succeeding foliage leaves (Avery, 1930). Its diameter is about 1.5 mm.; in etiolated plants it may attain a length of 6 cm. or more; but in plants grown under normal conditions, it seldom attains a length of more than 1 to 2 cm. The coleoptile is devoid of green pigment if grown in darkness and develops chlorophyll only sparingly under the influence of light. Some varieties of oats seedlings when grown in the dark develop an elongated first internode (a stem portion termed "mesocotyl" in the older literature).

In transverse section, the lower part of the coleoptile appears as in Fig. 21; a longitudinal median section through the embryo divides the coleoptile into two symmetrical halves, as indicated by the broken line in this figure. Embedded in the parenchyma tissue in either half are the two vascular bundles; these

end blindly just below the apex. The slightly dorsiventral structure of the coleoptile was observed by Rothert (1894), who designated the side facing the seed dorsal; the other, ventral, The greater part of the coleoptile consists of elongated parenchyma cells.

The shape of the 2 mm. long tip portion of the coleoptile is shown in Fig. 22. *A* is a section in the plane of symmetry; and

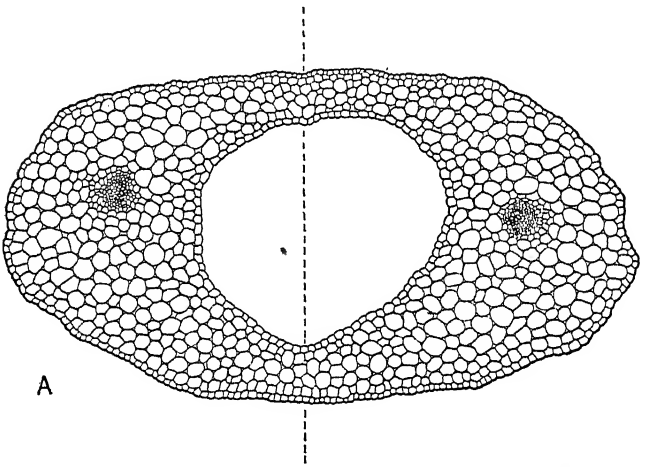


FIG. 21.—Drawing of a transverse section through an *Avena* coleoptile. *A*, cellular structure and position of vascular bundles at lowermost end of a mature coleoptile, × 40. *B*, cellular structure of the narrow portion at the base of a coleoptile 2 mm. in length. The number of cells is the same in the transverse sections of young and old coleoptiles.

B, perpendicular to the plane of symmetry. The dorsiventrality is obvious at the tip; on the ventral side a small slit, or pore, is formed, through which the foliage leaf emerges from the coleoptile (Fig. 23). The upper region between the pore and the tip is made up of small isodiametric cells with large nuclei, dense cytoplasm, and small vacuoles. This tip region, as is mentioned elsewhere, is far more sensitive to light than the lower zones.

Distribution of Growth.—The Avena coleoptile is an organ of limited growth. The number of outer epidermal cells remains the same throughout the growth period; *i.e.*, no cell division takes

place during the growth of the seedling (Figs. 23, 24, and 25). Their rate of elongation, therefore, is proportional to that of the coleoptile as a whole (Avery and Burkholder, 1936). The underlying parenchyma and inner epidermal cells increase their number 2.9 times on the average (Fig. 25). Of all the cell layers, the greatest number of cells from the tip to the base of the organ is in the subepidermis, and each successive layer inward has fewer cells. Most of the cell division takes place before the coleoptile is 1 cm. long, or about one-fourth its final length (Fig. 25); it is about evenly distributed throughout the whole length of the

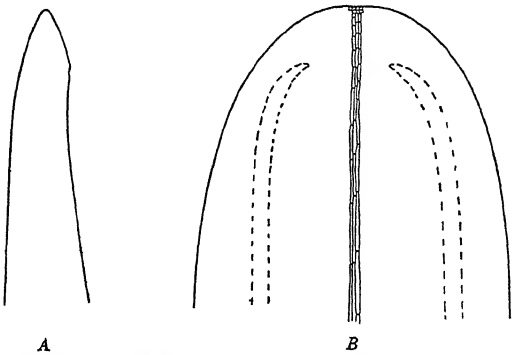


FIG. 22.—Diagrams of longitudinal sections through the tip of the *Avena* coleoptile. *A*, section through the narrow dimension, which is indicated by the dotted line in Fig. 21*A*. *B*, view through wide dimension with position of vascular bundles shown by broken lines. Note in detailed strip the increasing length of epidermal cells below the ends of the vascular bundles.

organ, except for the zone between the pore and the tip where there is no division whatsoever. Cell division and cell elongation take place only in the direction of the long axis of the organ; *i.e.*, growth is *polarized*. From a length of 1 cm. to maturity, the coleoptile grows in direct proportion to the elongation of its cells. The course of growth is influenced, of course, by temperature, humidity, and light.

The distribution of growth in the Avena coleoptile has been investigated by Rothert (1894), duBuy (1933), and more recently Avery and Burkholder (1936). The lower half of the embryonic coleoptile elongates to make up approximately three-fourths of the total length of the mature coleoptile, while the upper half of the embryonic coleoptile elongates to form the upper one-fourth of the mature coleoptile. Although growth is taking place

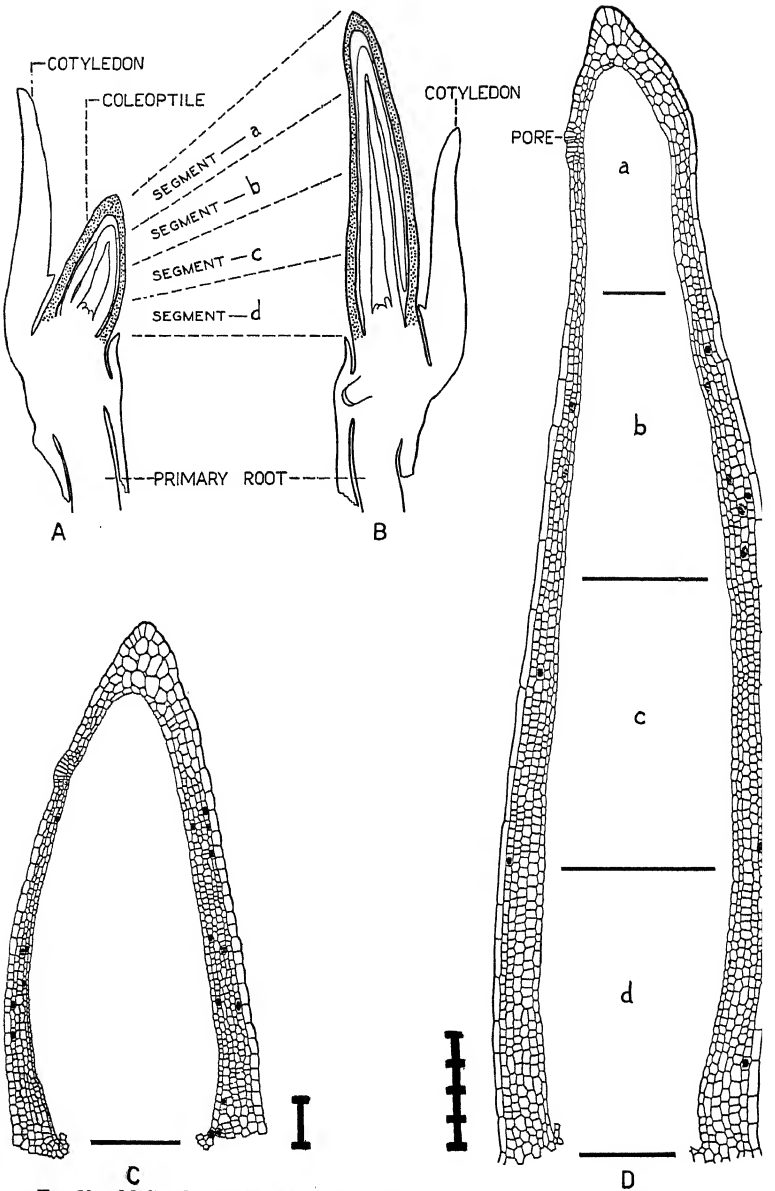


FIG. 23.—Median longitudinal sections of *Avena* coleoptiles at different stages of development. *A* and *B*, diagrams through germinating embryos, × 11. *C* and *D*, detailed drawings of coleoptiles, 1.62 mm. and 4 mm. in length, respectively, × 40. Mitoses are evident throughout. From *D* to maturity, the 1 mm. segments *a*, *b*, *c*, and *d* elongate to the dimensions indicated by the diagram in Fig. 24. The heavy line beside *C* and *D* indicates the relative length of the coleoptile, to be compared with the heavy line in Fig. 24. (*After*

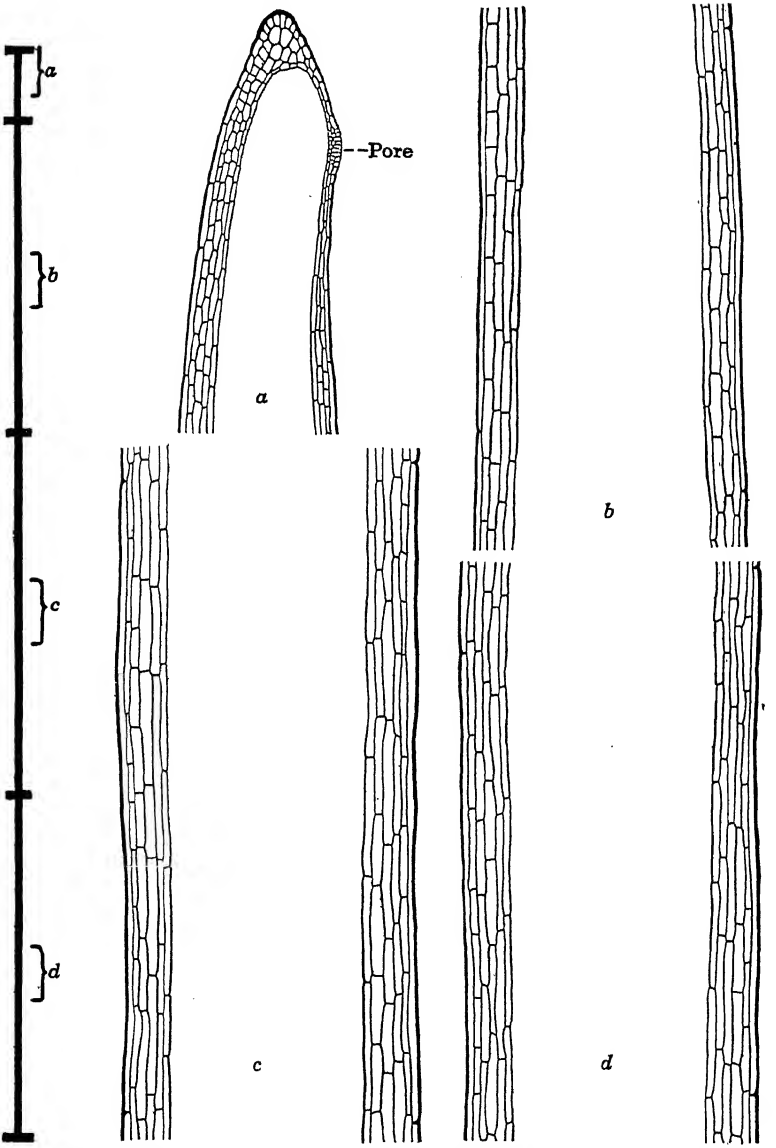


FIG. 24.—Detailed drawings of cells in longitudinal sections from different levels of a mature *Avena* coleoptile. The heavy line at the left indicates the relative length of the marked segments designated *a*, *b*, *c*, and *d* in proportion to the over-all length of the coleoptile. Compare with Fig. 23. The drawings represent part of the apical end of segment *a* and small portions from the middle of segments *b*, *c*, and *d*. Compare the small size of the cells near the apex with the elongated cells in the lower portions. Note the extreme length of the epidermal cells. (*Adapted after Avery and Burkholder*, 1936.)

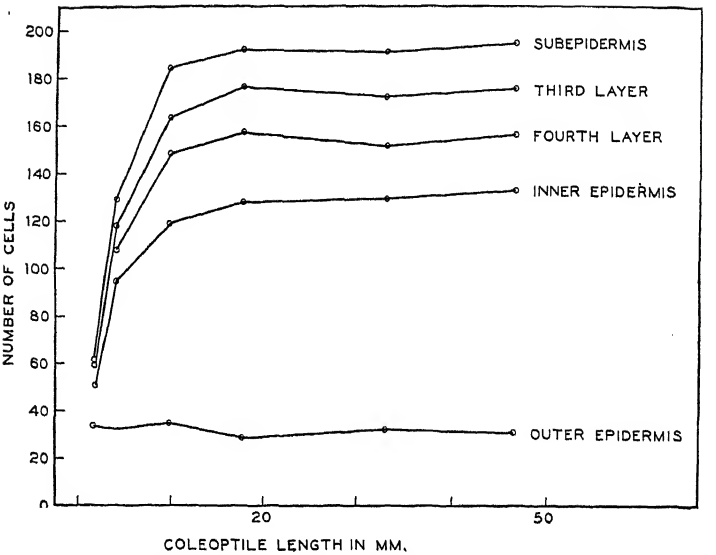


FIG. 25.—Graph showing the number of cells from tip to base in the different layers of the *Avena* coleoptile, at six stages in its growth. The outer epidermal cells do not increase in number, while the cells of the other layers multiply rapidly in the early period of development and then remain about constant in number. A definite gradient of cell-division intensity, decreasing inward from the subepidermis to the inner epidermis, is apparent for the first quarter of the growth period. During the last three quarters of its growth period, the increase in length of the coleoptile is proportional to the elongation of its constituent cells. (*From Avery and Burkholder*, 1936.)

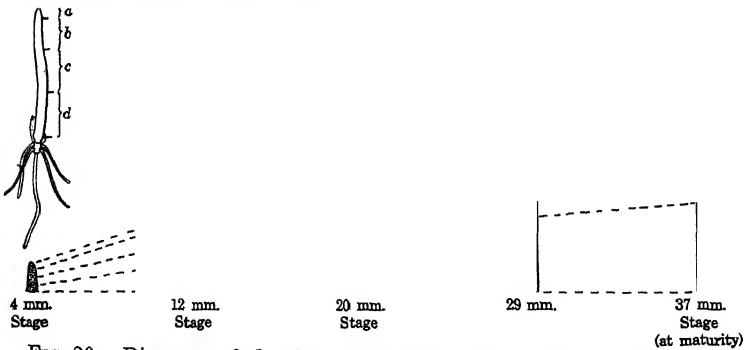


FIG. 26.—Diagrams of the *Avena* coleoptile to show shift in position of the zone of maximum growth intensity at different stages of development. Coleoptiles 4 mm. in length were marked into four 1 mm. segments (*a*, *b*, *c*, *d*) and the length of each was measured in several later stages of growth. The density of the dots indicates relative growth intensity. Note that the region of maximum growth shifts from the base in the young coleoptile (12 mm. stage) to the apical region in a maturing coleoptile. (*Adapted after Avery and Burkholder*, 1936.)

rapidly throughout most of the coleoptile while it is young, the region of greatest elongation is basal (Fig. 26). As the coleoptile nears maturity, growth slows down throughout its length, ceases at its base, and becomes relatively greater near its apex. At the time the foliage leaf bursts through, all basal growth has ceased, but a localized region of slow elongation at the tip below the pore may persist for as much as two or three days after the leaf bursts through the coleoptile. These same facts apply, in general, to the coleoptile of Triticum, on which similar observations were made.

Light-growth Reaction.—The inhibiting effect of light upon the rate of growth in plants was recognized in the older plant phys-

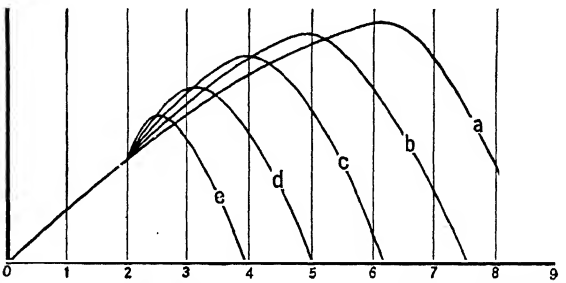


FIG. 27.—The course of growth in the *Avena* coleoptile in different intensities of light. Ordinate: growth; abscissa: 12-hour periods. *a*, growth of coleoptiles in weak light for 4½ days; *b–e*, growth of other coleoptiles kept for 1 day in the same weak light as in *a*, and then transferred to different intensities of light, increasing successively from *b* to *e*. Greater light intensity shortens the period of growth and causes the size of coleoptiles to be smaller at maturity. (*After Sierp*, 1918.)

iology literature (deCandolle, 1832; Sachs, 1874). Since the time when Blaauw (1914) attempted to use light-growth reactions as the foundation for a theory of phototropic curvatures, the role of light in growth has been the subject of many investigations.

Although Blaauw did not perform any experiments on the light-growth reactions in the Avena coleoptile, such investigations were carried out by Vogt (1915), Sierp (1921), Lundegardh (1921, 1922), Koningsberger (1922), Renner (1922), Brauner (1922), Erman (1923, 1930), Went (1926, 1928*a*), Dillewijn (1925, 1927*a*, *b*), Pisek (1926), Beyer (1926, 1927*a*, *c*, 1928*b*), Priestley (1926*c*), Gradmann (1930), Bergann (1930), Nuernbergk and duBuy (1930), Cholodny, (1931*d*, 1932*b*, 1933*a*), and duBuy (1933). Sierp (1918) studied the development of the Avena

coleoptile in darkness and when subjected to varying amounts of light. As may be seen in Fig. 27, he found that the rate of growth of the coleoptile is temporarily increased with increasing amounts of light. The point of maximum growth is reached sooner, and the final size of the coleoptile is not so great under conditions of increasing light.

Van Dillewijn (1927*a*) illuminated the Avena coleoptile by placing a lamp vertically over the plant; the light was reflected horizontally on to the experimental object by three oblique mirrors. He noted the influence upon the rate of growth of continued illumination as well as short periods of illumination with differing amounts of light. In some experiments only the tip was illuminated, in others, the subapical zones; or the entire coleoptile was supplied with light. Light-growth reactions appeared in all cases. The reactions were sharply defined when definite zones, near the tip, were illuminated for a short time with a definitely determined amount of light. After a latent period there occurred a depression of growth during the course of which two types of response could be distinguished, one of short, the other of long duration (Sierp, 1921). In the short reaction, the maximum depression of the growth rate was reached after ½ hour; in the long reaction, on the other hand, after 1½ hours. The long reaction could be observed only when the tip was illuminated (Went, 1928*a*: *tip response*); the short reaction, when the basal zones were illuminated (Went: *base response*). Growth was accelerated again after this retardation. When the entire coleoptile was illuminated, these effects were summated. When illuminated plants were darkened, Sierp (1918) found that a dark-growth reaction took place also.

Geo-growth Reaction.—The question whether gravity can produce fluctuations in the growth of the Avena coleoptile in a manner similar to that brought about by light has been investigated with contradictory results. Zollikofer (1921) reported continually changing rates of growth in response to stimulation by gravity; while Koningsberger (1922) observed no geo-growth reaction during continued rotation on the clinostat, but a growth-promoting effect was produced by gravity in both erect and inverted coleoptiles. The dorsiventrality curvatures, however, mentioned elsewhere, can easily disguise growth changes when the Avena coleoptile is clinostated. With this source of error

removed, Bremekamp (1925) and Dolk (1929*a*) showed that no geo-growth reactions appear in the Avena coleoptile. Navez and Robinson (1932*b*) came to the same conclusion.

Growth Substances and Normal Growth.—As has been mentioned previously, Paál (1918) showed that growth substance is being formed continuously in the nonilluminated coleoptile tip, whence it migrates into the more proximal portions of the coleoptile and promotes growth.

Rothert (1894) and Stark (1917) showed that the removal of the coleoptile tip produces a retardation of growth in the coleoptile stump, a fact that Söding confirmed when he investigated this same question (1924, 1925, 1929). The rate of growth (Table 3)

TABLE 3.—GROWTH IN LENGTH OF NORMAL AND DECAPITATED AVENA COLEOPTILES

The figures in the table are average values from Tables I to III of Söding, 1925 (p. 589)

Treatment	Increase in the first 5 hr.	Increase in the following 13 hr.
A. Decapitated	0.63	2.57
B. Decapitated, the tip replaced, and again removed after 5 hr	0.94	1.65
C. Intact control plants	1.49	3.40

in the first 5 hours after decapitation was only 42 per cent of that in normal seedlings. Furthermore, the rate of growth of the coleoptile stump was increased about 49 per cent in the first 5 hours when the removed tip was again replaced. Söding's experiments showed that the rate of growth of normal plants is not reached in decapitated plants in the first few hours, even with their tips replaced, probably because the transport of growth substance is retarded by the wound. After 10 to 14 hours, even without replacing the tip, the rate of growth of decapitated coleoptiles became about the same as that of normal seedlings. This increase in growth was brought about by "physiological regeneration" of the tip, which produced about the same amount of growth substance as the normal.

It is clear from this that a substance is dispersed from the tip which promotes growth in the basal region. If, instead of replac-

ing the tip after decapitation, one covers the wound with agar containing growth substance, the rate of growth can be increased far beyond the normal (Fig. 1) (Went). It has been found that when the growth-substance content of the agar amounts to 100 WAE (Boysen Jensen, 1933*a*), the coleoptile stump surpasses the enclosed leaf in growth, which normally never occurs. Söding (1929) investigated different portions of the coleoptile for growth substance and found that the amount decreased greatly from tip to base. This observation has been confirmed by the work of Thimann (1934).

From these and other experiments, it has been concluded in the past that growth substance is formed exclusively in the tip under normal conditions and that it migrates from there into the more proximal portions of the coleoptile where it stimulates growth. In view of the upward movement of growth substance which has been demonstrated in certain plants by Zimmerman and Wilcoxon (1935), it appears equally probable that the hormone or its precursor is being formed in the endosperm (Cholodny, 1935*b*) and moved upward in the vascular system to the tip, from which point it is dispersed downward. In fact, Pohl (1935) concludes from a series of important experiments that the coleoptile tip does not produce growth substance but can only activate the reserve stored in the endosperm. The phenomenon of "physiological regeneration" apparently could be explained by this interpretation. Further confirmatory evidence is found in the observation that physiological regeneration (Söding, 1929) takes place just as vigorously whether the coleoptile is decapitated at the tip or several millimeters below and Heyn (1935) has found that physiological regeneration does not take place when the coleoptile is separated from the food stored in the seed.

The hypothesis that the decrease in rate of growth after decapitation may be caused by lack of growth substance has been disputed by Priestley (1926*d*) and by Tetley and Priestley (1927). When the coleoptile is decapitated, water exudes from the cut surface; this loss of water is, according to Priestley, the essential reason for the retardation of growth, and he contended that retardation must persist until the supply of water is rendered normal again by healing of the wound. The promotion of growth by replacement of the tip was explained by partial closing of the wound. It may be said here that Priestley's explanations

are no longer tenable in the light of the more recent discoveries concerning the role of growth substance.

A question of importance is whether growth takes place in the Avena coleoptile when growth substance is completely absent. As shown by Söding's experiments, some growth takes place in the first 5 hours after decapitation, but Dolk (1930) showed that this occurs only because of the growth substance still

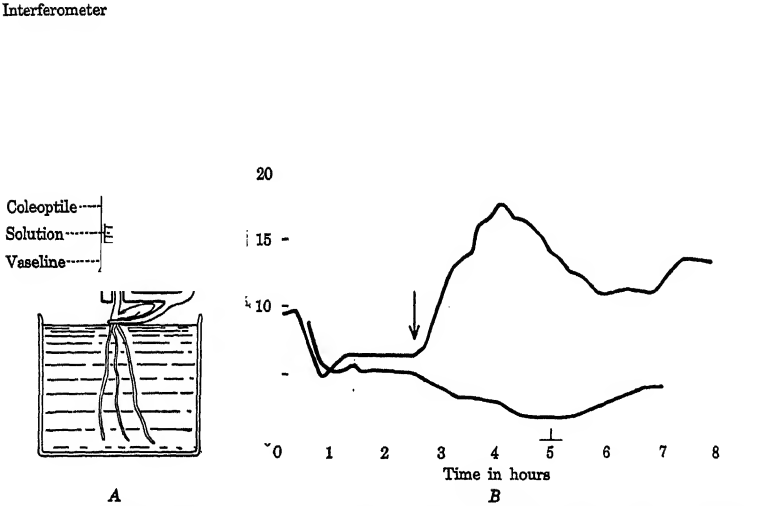


FIG. 28.—Stimulating effect of growth hormone (acidulated pollen extract) upon growth of a decapitated *Avena* coleoptile. *A*, diagram showing culture chamber in which coleoptile is grown immersed in solution. *B*, graph showing the increased rate of growth of a coleoptile treated with growth-hormone solution at the time indicated by arrow, as compared with a control plant (lower curve) which continues its growth in water. Growth was measured interferometrically. (*After Laibach and Kornmann*, 1933*a*.)

present in the coleoptile stump. If the coleoptile is decapitated again 2 hours after the first decapitation, its growth ceases almost completely but can be renewed by supplying growth substance. From these experiments it may be concluded that normally no growth substance is formed below the tip of the coleoptile and that the growth substance present in the stump at the time of decapitation is gradually used up. In any case, without growth substance there is no growth.

An interesting technique was developed by Laibach and Kornmann (1933*a*) to demonstrate the accelerating effect of growth substance (extracted from pollen) upon growth in length of the decapitated Avena coleoptile (Fig. 28).

Went (1928*a*) suggested reasons for the distribution of growth in the Avena coleoptile, stating that the rate of growth in the

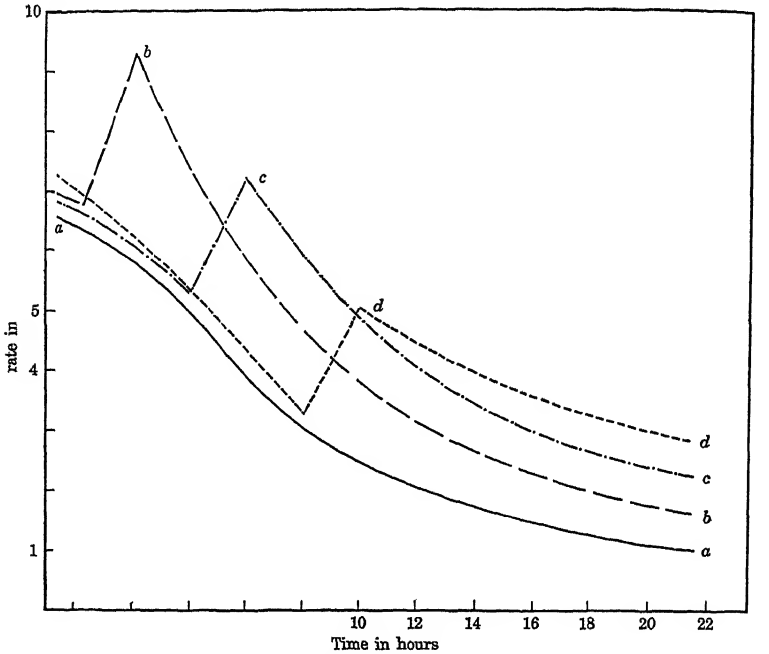


FIG. 29.—Growth rate of the upper 10 to 15 mm. zones of *Avena* coleoptiles with different amounts of growth hormone. *a*, the normal course of growth; *b*, growth of a group of coleoptiles to which auxin paste was added at an early stage; *c* and *d*, other groups of coleoptiles to which auxin was applied at later stages in growth. (*After Went*, 1935*c*.)

basal portion is limited by the failing supply of growth substance; on the other hand, growth in the tip is limited by the lack of organic material (supplied by the seed) which is necessary for cell elongation. The rate of growth reaches a maximum at that point where both food and growth substance are present in sufficient amount, and the water supply is adequate. DuBuy showed (1933) that growth in the coleoptile is gradually retarded when the endosperm is removed; aging is also mentioned as one

of the factor complexes significant in its growth. Went has discussed the subject in a later paper (1935*c*) and concluded that growth substance is a limiting factor in the elongation of the coleoptile during its later stages of development. Artificially increasing the auxin supply in a coleoptile accelerates the growth rate either directly by promoting growth or indirectly by preventing senescence (Fig. 29). With a supply of food available, the addition of auxin brought about a revival of growth in the basal portion which had ceased to elongate; on the other hand, when the food supply was removed, further additions of auxin showed no growth-promoting effect.

From the foregoing observations it seems clear that the rate and distribution of growth in the normally developing coleoptile are regulated by the supply of growth substance.

Growth Substances and the Light-growth Reaction.—Went (1926) and van Dillewijn (1927*a*) conjectured that the growth reactions produced by complete illumination of the tip zone are the result of changes in the amount of growth substance given off, and Went (1928*a*) actually found a decrease of about 18 per cent in the amount of auxin given off when the tip was illuminated with 1,000 meter-candle seconds. According to duBuy (1933), weak blue light produces no change in growth-substance supply, and even strong white light (with heat and some of the red removed) may have no effect; on the other hand, white light plus the heat radiation decreases the supply of growth substance.

General illumination of the lower zones of the coleoptile also produces light-growth reactions, as mentioned earlier, but it is not possible at this time to offer a satisfactory explanation of the phenomenon. Further data are discussed under light-growth reaction in the stems of seedlings. That the influence of light on growth depends upon the kind of growth substance present has been shown by van Overbeek (1936*a*). When auxin *a* is applied unilaterally in agar blocks to Avena coleoptiles, curvature is less under illumination with white light, than in darkness; when 3-indole acetic acid is similarly applied, no difference in growth is observed in darkness or in light.

Foliage Leaves.—The presence of growth substance has been demonstrated in buds and foliage leaves of several species of dicotyledons (see chapter on the occurrence of growth substances), and in Nicotiana (Avery, 1935) it has been shown that

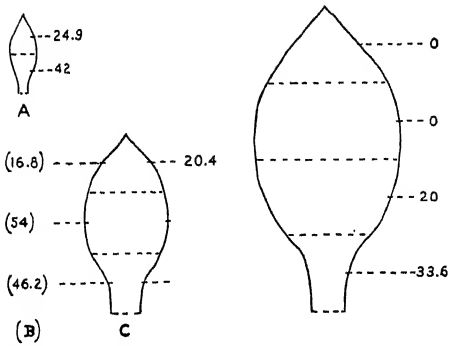


FIG. 30.—Diagrams of *Nicotiana* leaves showing growth-hormone content (expressed in plant units) at different ages and in different portions. *A*, young leaf. *C* and *D*, older leaves from plants grown in a greenhouse. *B*, leaf of same age as *C*, but kept in dark for 10 days, followed by 1 day in the light. The auxin concentration gradient shown in *A* and *C* is due to accumulation in the midrib and movement toward the base of the leaf. In contrast, leaf *B* shows less accumulation at the base (data in parentheses). Note disappearance of growth hormone at the distal end of the older leaf, *D*. (*After Avery*, 1935.)

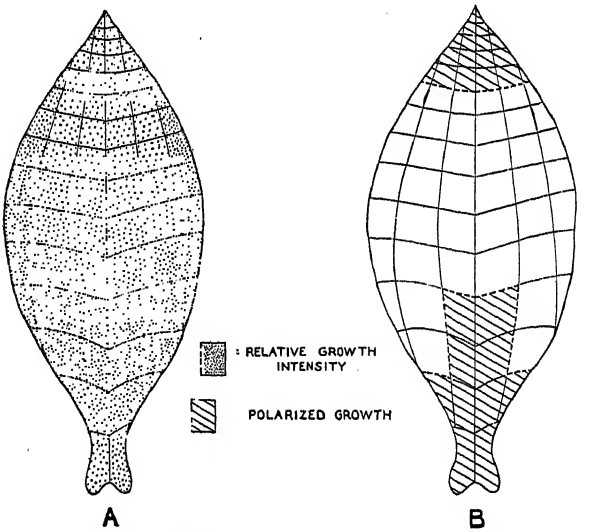


FIG. 31.—Growth of the *Nicotiana* leaf. *A*, showing greatest growth intensity (*localized growth*) in marginal and basal regions, as indicated by the density of stippling. *B*, the segments indicated in the distal and proximal portions of the leaf show a relatively greater increase in length than in width. While this *polarized growth* is not pronounced at the apex toward the end of the growth period, it is very striking at the basal end of the leaf, where it is correlated with higher concentrations of growth hormone. (*After Avery*, 1935.)

the concentration is greater in young leaves, tending to decrease as the leaves mature. It has been shown, also, that there is a definite concentration gradient from the tip to the base of a leaf, the concentration being low at the distal end and increasing toward the base (Fig. 30). The increase toward the base is due to the accumulation of growth substance in the proximal end of the midrib and is correlated with greater longitudinal growth ("polarized growth") of the midrib in this region. Inasmuch as the application of growth-substance paste (lanolin method, Laibach, 1933*b*) to large veins brings about a bending (differential growth) response, it may be assumed that it is the agent responsible for promoting the normal growth in length of the midrib and larger lateral veins in the leaf; hence, growth substance is responsible, at least in part, for the normal growth pattern exhibited by the leaf (*cf.* Figs. 30 and 31).

Axial Parts: Hypocotyls, Internodes, and Flower Stalks. *Distribution of Growth.*—Rothert (1894) investigated the distribution of growth in the hypocotyls and epicotyls of dicotyledonous seedlings. In seedlings with epigeal cotyledons the hypocotyl usually elongates first. Enlargement of the growing point above the cotyledons begins only after the growth in length of the hypocotyledonary axis is completed. As long as the hypocotyl is very short, it grows throughout its entire length; later the basal portion ceases growing, and a growth zone of a rather constant length (1 to 4 cm.) is established in its upper portion. Following cessation of growth in this region the epicotyl begins to develop. The distribution of growth in the epicotyl of Phaseolus is shown in Fig. 32.

The distribution of growth in stems with several elongating internodes is often quite complicated. It would not be of value to discuss this question at length here, since the significance of growth substance for these growth processes has not been investigated.

Very obvious light-growth reactions are exhibited by many seedling axes. According to Blaauw (1915), a decided retardation of growth appears in Helianthus after brief illumination; this is followed later by an increase in growth enduring for a short period. According to van Overbeek (1933), the rate of growth in the hypocotyl of Raphanus is decreased to about one-half by illumination.

The observations of Thimann and Skoog (1934), working with epicotyls of *Vicia Faba*, show that a growth-promoting substance is formed by the action of light upon the green portions of the

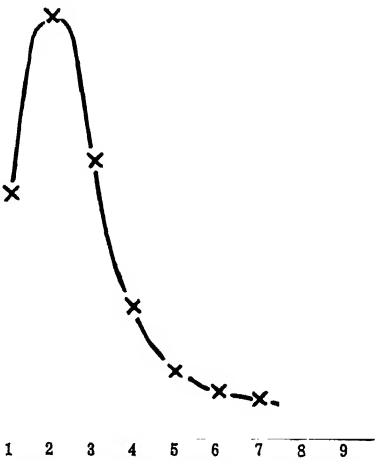


FIG. 32.—Distribution of growth in the seedling stem of *Phaseolus multiflorus* (hypogeal cotyledons) at 24°C. The stem was marked into nine 5 mm. zones, beginning at the level of the first foliage leaves. Ordinate: growth in millimeters in 24 hours; abscissa: 5 mm. zones. (*After Boysen Jensen.*)

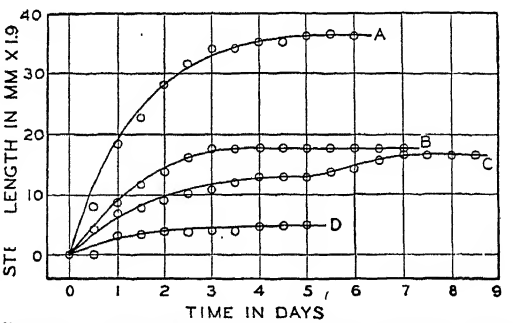


FIG. 33.—Growth of defoliated and decapitated plants of *Vicia Faba* in the light and in the dark, with and without growth hormone. *A*, growth in the dark, following the addition of 1600 units of growth hormone per cubic centimeter. *B*, growth in the light with the same amount of growth hormone as in *A*. *C*, growth in the light following the application of plain agar. *D*, growth in the dark with plain agar. (*Adapted after Thimann and Skoog*, 1934.)

plant. The rate of growth, however, is more rapid in darkness than in light in experiments where comparable amounts of growth substance are added (Fig. 33).

Growth Substances in Relation to the Distribution of Growth.— From the present evidence it is possible to distinguish two types of seedlings on the basis of the place of growth-substance formation in their hypocotyls. *Raphanus sativus* may be cited as an example of the first type (van Overbeek, 1933), where the growth substance is formed in the cotyledons and moves from these into the hypocotyl. If the cotyledons are removed, the rate of growth falls off rapidly. After a time, the hypocotyl begins to grow again in its upper zones because the growing point has begun to form growth substance. That it is a growth substance which influences the growth of the hypocotyl is clear, for when a block of agar containing growth substance is placed upon one of the petiolar stumps, a negative curvature in the hypocotyl is produced.

The hypocotyl of *Lupinus albus* behaves differently. That its growth rate is influenced by growth substance was shown by Cholodny (1926) by boring out the middle portion of a 3 cm. segment so that only a hollow cylinder remained. Its rate of growth was greatly lessened by this operation, but if Zea coleoptile tips were placed in the hollow region, approximately the same rate of growth was obtained as in untreated normal stems. The work of Dijkmann (1933) shows that growth substance is found throughout the whole growing zone and is probably formed throughout. There seems to be no center for production of growth substance, and decapitation does not produce an immediate retardation of growth. According to some unpublished experiments of Boysen Jensen, *Phaseolus multiflorus* belongs to the same type.

The later work of Dijkmann (1934) indicates that the growth rate in the Lupinus hypocotyl is proportional, within certain limits, to the growth-substance concentration.

According to van Overbeek (1933), the light-growth reaction of the Raphanus hypocotyl is not caused by decreased production of growth hormone. It is explained by assuming a change in the ability of the organs (perhaps the cell walls) to react to growth substance.

The rate of growth of various inflorescence stalks is influenced also by growth substance. Uyldert (1928) showed that the elongation of flower stalks of Bellis is retarded greatly by the removal of the inflorescence, but it could be increased again by

the addition of growth substance. The rate of elongation of decapitated flower stalks is increased by rhizopin, also (Nielsen, 1930*a*). Söding (1932*b*) showed that unilaterally applied Avena tips produce curvatures in flower stalks of *Heliopsis laevis*, *Cephalaria tatarica*, and *Muscari ramosum;* therefore, in these organs, also, the rate of growth is increased by growth substance.

That the growth hormone present in the young internodes and nodes of grasses influences their elongation may be concluded from the fact that growth substance from coleoptile tips increases

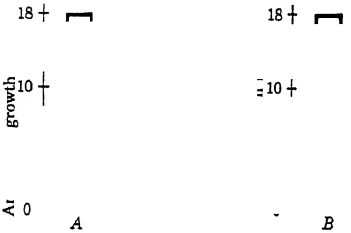


FIG. 34.—Comparison of the growth-hormone content and growth response of a normal race of *Zea mays* with the dwarf type "nana." *A*, coleoptile tips of the normal race yield almost double the amount of growth hormone given off by the dwarf "nana"; data from 400 plants of each type. *B*, growth curvature of coleoptiles of the normal race is about twice that of "nana" when a given amount of growth hormone is applied unilaterally in agar blocks; data from 200 plants of each type. (*After van Overbeek*, 1935.)

the growth of young internodes. It has been shown to produce cell elongation even in mature nodes (Schmitz, 1933).

The first internode (mesocotyl) in *Zea mays*, dwarf variety "nana," is appreciably shorter than the first internode in a normal race, although the coleoptiles are the same length. Van Overbeek (1935) has shown that the inhibited development of this first internode is due to the *destruction* of auxin, the amount produced being about the same in seedlings of nana and normal. A given amount of growth hormone applied unilaterally to the coleoptile stumps of normal and nana resulted in smaller curvature of the latter (Fig. 34), thus supporting the conclusion regarding destruction. This destruction is correlated with a greater catalase and peroxidase activity; hence van Overbeek concluded that the dwarf type of growth in this variety of maize must be due to a more active oxidation system. By raising the temperature of normal seedlings to 60°C. for one hour, thus increasing the catalase activity, it was possible to make the normal into dwarf,

i.e., to induce the destruction of auxin and obtain a short first internode.

Roots.—Roots are a continuation of the axial system of the plant but differ sharply from the shoot in structure and behavior. The region of primary growth in the root is so well-known that it is unnecessary to describe it here. With regard to the presence of growth substance in roots, there is considerable evidence indicating that it is formed by the root tip; lesser amounts are found proximally.

Wiesner (1881) made the statement that the growth of roots in contact with water is accelerated by decapitation. This observation was confirmed by Cholodny (1926); an increase in growth of 12 per cent took place after decapitation of the roots of *Lupinus angustifolius*. Then Bünning (1928) investigated the effect of decapitation upon the growth of the root; if the removed tip portion was not too long, there was a temporary retardation of growth in most roots, followed by an increase. Nielsen (1930*b*) also showed an increase in root growth as the result of decapitation.

To answer the question of how decapitation can produce an increase in growth, Cholodny assumed that the growth substance that is formed in root tips must be identical with that formed in coleoptile tips. Even though the growth substance increases the growth of the Avena coleoptile, it apparently retards the rate of growth of the root. If this is so, the removal of the root tip should bring about an increase in growth. Bünning (1927) concluded, on the other hand, that the growth changes described are to be construed as wound-growth reactions.

It has been demonstrated repeatedly that growth substance can influence the rate of growth of roots. Cholodny (1924) showed that decapitated roots of *Zea mays*, upon which had been placed the coleoptile tips of the same plant, grew 36 per cent less rapidly than decapitated roots without tips. This indicates that the growth substance of the coleoptile retards growth of the root. This conclusion was confirmed by Nielsen (1930*b*), who determined the growth increase of the root when the 2 mm. portion of the tip was first submerged in water and afterward in a rhizopin solution. He showed that the rhizopin completely inhibited the growth of roots of *Lupinus albus* and *Vicia Faba*, whether they were intact or decapitated. Moreover, the roots

were not permanently injured by the rhizopin, for if the rhizopin solution was replaced later by water, the roots immediately resumed their growth.

In contrast with these results, Gorter (1932) concluded that growth substance had no influence upon the growth of roots of Pisum and maize. The roots were decapitated, and agar blocks containing growth substance from the coleoptile tips of maize were placed upon the wound surface. The experiment included six Pisum roots (three with agar and growth substance, three with agar without growth substance) and three maize roots. When growth substance was added, the rate of growth in Pisum was greater, in two cases, than when it was not present. Growth was greatest in maize without added growth substance. The author concluded from her experiments that growth substance has no influence upon the growth of the root. Her data were too few to permit a final conclusion on this matter. To clear up the existing disagreement in reports of the different workers, Boysen Jensen (1933*c*) carried out further experiments on the influence of growth substance upon the rate of growth in roots of *Vicia Faba*. In the first of these studies the methods of Nielsen were used. The rate of growth was determined for root tips, some of which were immersed in pure water and others in a growth-substance solution containing 2 WAE per 100 cc.; in the latter case, growth decreases by about half. The use of Gorter's method also showed that growth substance influences the rate of growth of the root, but with her procedure it was necessary to use far higher concentrations of growth substance. In this method, the roots were decapitated 1.25 mm. back of the tip, and agar blocks were placed upon the wound surfaces, either without or with growth substance in concentrations of 25 to 50 WAE per 100 cc. The rate of growth was reduced about one-half by this concentration of growth substance (see also Cholodny, 1933*b*; Navez, 1933*b*).

Kögl, Haagen-Smit, and Erxleben (1934, Mitt. XII) have shown that 3-indole acetic acid when added to the culture solution in concentrations of 0.01 to 1 mg. per liter inhibits root growth; auxin *a* and *b* have similar effects. The work of Meesters (1936) has shown further the inhibiting influence of 3-indole acetic acid on the growth of root hairs and roots of Agrostemma. Growth in length of the root hairs was retarded

by about 20 per cent in the presence of 0.5 mg. of 3-indole acetic acid per liter; almost complete inhibition of root elongation occurred with the same concentration of the hormone. Other solutions of the same pH value, obtained by the addition of acetic acid, did not show any inhibiting effects.

It has been determined with certainty, therefore, that the rate of growth of roots is retarded by the addition of growth substance, and from this it might be concluded that growth substance is not necessary for the growth of roots. In support of this interpretation is the fact that ageotropic roots, which can be obtained by treating the seed with eosin or erythrosin (Boas and Merkenschlager, 1925), often possess no demonstrable amount of growth substance (Boysen Jensen, 1934), although the rate of growth is not decreased.

The evidence from normal distribution of growth substance in roots makes another interpretation equally plausible. Boysen Jensen (1933*b*) and Thimann (1934) both have shown that a concentration gradient exists at the growing end of the root, the tip possessing the most growth substance, and the concentration falling off in a proximal direction. From this it might be concluded that growth substance does take part in root growth and that elongation of the root is taking place in the region of optimum concentration. If this is the case, the optimum concentration for root growth must be very low. Why the root and shoot behave differently in the presence of a given concentration of growth substance remains to be explained. [Czaja (1935*b*) discusses a possible explanation based upon the direction of streaming of growth substance in roots.]

Lower Plants.—Heteroauxin is produced by many lower organisms, *e.g.*, *Aspergillus niger*. This substance has a remarkable effect upon the rate of growth of the Avena coleoptile, and it is important to determine whether it has any demonstrable physiological significance for the growth of Aspergillus itself.

Boysen Jensen (1932) showed that if Aspergillus is cultivated upon a glucose-nitrate solution, growth substance cannot be demonstrated either in the fungus mycelium or in the culture substratum. This would indicate that growth substance is not a necessity for the growth of Aspergillus. Whether it has any influence upon the growth of this organism was determined in the following way: *Aspergillus niger* was grown upon a glucose-

nitrate-citric acid solution, in some experiments without growth substance, in others with it present in concentrations of 0.9, 9.0, and 21.0 WAE per 100 cc., respectively. After being cultured for 11 to 14 days at a temperature of 16°C., the mycelium was removed, dried, and weighed. It was found that the addition of growth substance always resulted in retardation of growth—in one case by as much as 50 per cent; in other cases, by significantly less. This is in agreement with the results of Nielsen and Hartelius (1932), who found that rhizopin was without influence upon respiration or the production of dry matter in *Aspergillus niger* (see Nielsen, 1931*a*). Bünning (1934*a*, *b*) also found no furthering influence of the ether-soluble hormone of Aspergillus upon production of dry substance in this fungus. Bünning, Schopfer (1935), and others have shown the growth-stimulating effect of other substances on lower organisms. Schopfer reported that extracts from wheat embryos, orchid and other pollens, etc., stimulate the growth of numerous Mucorineae. Pure crystallized vitamin B_1 promotes the growth of Phycomyces in such small amounts as 0.0005γ per cubic centimeter of the culture medium. However, the extracts with which Schopfer has been working contain active substances which are not to be confused with the growth substances treated at length here.

Animal Cells.—Since substances capable of promoting growth in plants can be extracted from animal sources, it is of interest to find out whether these substances have any influence on animal growth.

According to the investigations of Fischer, the growth of heart fibroblasts in tissue cultures is not increased by auxin *a* or *b* (see Kögl, Haagen Smit, and Tönnis, 1933, Mitt. VIII), nor is the metamorphosis of tadpoles influenced by the addition of auxin (Kögl, Haagen Smit, and Erxleben, 1933, Mitt. VII; Sylvén, 1933). Navez and Kropp (1934) obtained similar negative results when they applied the plant-growth hormone to crustacean eyestalks; *i.e.*, there was no activation of the chromatophores.

Plant-tissue Culture.—LaRue (1935) removed pieces of the embryos of half-grown seeds of Taraxacum, Lycopersicon, Lactuca, etc., and cultured them on nutrient agar. White's (1934) culture solution was used but without the yeast extract. In Lactuca, complete plants developed from 0.5 mm. pieces of embryonic hypocotyl. Successful growth took place only in

cultures with 3-indole acetic acid (heteroauxin) (1 part: 20,000,000).

Growth Substance in Relation to Cell Division.—The role of hormones in promoting growth by cell enlargement has been established by numerous investigations upon diverse plant materials. A limited number of observations have led to the suggestion that growth substances may also influence cell division in plants.

Laibach, Mai, and Müller (1934) obtained an ether-soluble, thermostable extract from orchid pollen and urine which when applied to the stems of Coleus and Tradescantia brought about increased frequency of cell division leading to callus formation. Further work (Laibach and Fischnich, 1935*a*) has shown that 3-indole acetic acid stimulates cell division in the epicotyls of *Vicia Faba*. More recently still, it has been found that application of purified auxin *a* and 3-indole acetic acid to the upper ends of decapitated *Helianthus* seedlings caused growth in thickness by cambial division (Snow, 1935*b*). In a report of new experiments and a review of the literature, Jost (1935*b*) has pointed out the stimulating effect of relatively high concentrations of various substances upon cell division in the pith of *Vicia Faba*, the main roots of Lupinus, etc. Popoff (1933) studied the influence of growth-substance extracts which were obtained from the coleoptile and other parts of Zea seedlings and added to cultures of *Euglena gracilis*. It was reported that oxidation processes, cell division, and germination of the cysts were promoted by dilute concentrations of the extracts.

Other workers have postulated the existence of other special hormones for cell division. The role of certain substances (*e.g.*, bios) which apparently do not belong in the same category with the auxins has been described by many investigators (Wildiers, 1901; Miller, 1930; Schopfer, 1935; Dagys, 1934; Kögl, 1935, Mitt. XIV; etc.).[1] The precise way in which the auxins and these other substances may regulate growth by means of their influence upon cell division has not yet been satisfactorily explained.

THE MECHANISM OF ACTION OF GROWTH SUBSTANCES

In the foregoing survey of the discoveries concerning the effect

[1] See supplementary bibliography on p. 247.

of growth substances upon growth, we have found that they influence cell enlargement ("stretching growth") in diverse kinds of higher plants. The subject is not so simple because in some organs growth is stimulated, while in others it is inhibited by the presence of growth substance. In coleoptiles and portions of stems, growth is increased, while in roots, it is retarded; it is possible that growth substance may not be necessary for root growth, though its role in the neoformation of roots has been observed. Moreover, it appears to have little significance for the vegetative growth of Aspergillus and perhaps many other lower plants. The next step is to determine in what way growth substances exercise their growth-promoting effect.

From investigations carried out by Went (1928*a*), van der Weij (1932), and duBuy (1933) on the transport of growth substance, it can be seen that the hormone is actually used up in the growth of the Avena coleoptile. Van der Weij has shown that if two agar blocks, each with the same growth-substance concentration, are placed on either end of a coleoptile cylinder 2 mm. long, a decrease in growth substance takes place in the upper block, but no increase can be demonstrated in the lower block. The most likely explanation of this and numerous similar observations is that it is consumed in growth.

Growth Substance and the Cell Wall.—Up to the present, close quantitative relationships between consumption of growth substance and growth have not been demonstrated, and the present evidence is insufficient to prove that it participates stoichiometrically in the growth of the cell wall. Nielsen (1930*a*, *b*) showed that its effect is very great in proportion to its weight; and according to Kögl (1933, Mitt. III; see also Kögl, 1933*a*), a curvature of 10 deg. results in the Avena coleoptile from the action of less than one 50-millionth milligram of auxin *a* or *b*. Thimann and Bonner (1933) have computed that 2.31×10^{11} growth-substance molecules can produce a deposition of 6.8×10^{16} $C_6H_{10}O_5$ molecules of glucose residues for the cellulose micelles, *i.e.*, that one growth-substance molecule is active in the formation of 3.0×10^5 $C_6H_{10}O_5$ molecules. Although these numbers are approximations, they are entirely adequate to show that growth substance does not participate as a "building stone" of the cell wall; it must influence the growth of the cell in some other way. At this point in the discussion, it may be well to

review briefly certain studies on the composition and the microscopic structure of the cell wall.

According to the investigations by Thimann and Bonner (1933), the dry matter of the cell walls of the coleoptile contains about 42 per cent cellulose, the remainder being made up of hemicelluloses, pentosans, and pectins. Recent observations have indicated that the cell wall consists of two different elements, namely, micellae, *i.e.*, little rods, which are probably crystalline, and an intermicellar substance, which fills the spaces between the micellae (Anderson, 1935). According to Heyn (1933*b*; see also Kolkmeijer and Heyn, 1934), dehydration shrinks the cell wall to about one-third of its diameter when wet. Söding (1934) has assumed that the intermicellar substance is of greater volume than the micellae and that it is made up of a viscous, colloidal substance. Heyn (1933*a*, *b*, 1934*b*) has studied the cell walls of the coleoptile under the microscope and by means of the Röntgen spectrograph. He states that the cell walls are smooth in intact cells, although the inner layers become wrinkled when dehydrated or released from tension; the outermost layers of the external wall of the epidermis would be shorter than the inner layers when dried out. By means of the Röntgen spectrograph a difference can be found between younger and older cell walls. Heyn concluded the following from these experiments:

> If one assumes that the cellulose macromolecules of the young cell wall have not yet taken on the crystalline form described above, then the important role of the water held in these walls becomes understandable. In older cells (fibers), aging of the walls is accompanied by progressive dehydration, while more macromolecules are continually taking on this crystalline form, until finally a pure cellulose pattern is obtained. When young walls, consisting of macromolecules, are dried, much more bound water remains between the single molecules not in crystalline form.

Cell-wall Extensibility.—When considering the physical characteristics of the cell wall, its extensibility is of interest. Extension can be either reversible (*elastic extensibility*) or not reversible (*plastic extensibility*). According to Pringsheim (1932), one cannot make a sharp distinction between these two types; the amount of wall substance is not changed in either case. Measurement of the degree of extensibility in plant organs is a difficult problem; plant cells can change their length easily without

addition of wall materials. The walls can change their elastic and plastic properties by hardening, by increasing wall substance, and in many other ways.

Methods for Measuring Extensibility.—A technique by which cell-wall extensibility can be measured may be described briefly as follows: A decapitated coleoptile or flower stalk can be suspended perpendicularly, and the changes in length in a definite region may be determined when a weight, for example, 2 to 10 g., is attached to the lower end of the organ. The plant part being tested must not be turgid, because the change in length resulting from a definite pull on a turgescent coleoptile is only a small fraction of the change in length of a coleoptile which is in a state of plasmolysis. For plasmolyzing, one may use a 50 per cent glycerin solution. The measurements are made with the horizontal microscope. If the original length of the region marked previously on the coleoptile is termed a, the length after attachment of the weight b, and after removal of the weight c, then the total increase in length (extension) is $b - a$ (elastic and plastic extension), the remaining increase in length, $c - a$ (plastic extension). If weights of 2 g. are used on the oat coleoptile, the extension that remains is negligible; therefore, only elastic extensibility is measured (Söding, 1931). When a weight of 10 g. is used (Heyn, 1931*b*), the increase in length remaining after the weight is removed ($c - a$) is about 30 per cent of the total length increase ($b - a$).

Heyn (1931*b*) used the following method to measure plastic extensibility: Excised coleoptiles, with the primary leaf removed, were fastened at one end and placed in a horizontal position (Fig. 35). A weight of 250 mg. was placed upon the free end for a given length of time. After the weight was removed, the size of the bend formed in the coleoptiles was determined. This curvature was used as an index of plastic extensibility.

It is possible, also, to measure the extension of the cell wall which is produced by osmotic pressure (Schmid, 1923). The plant part is first placed in water and afterward plasmolyzed. The difference in length before and after plasmolysis is a measure of the extension of the cell wall due to osmotic force.

Hypotheses on the Method of Action.—Since growth substances may have a controlling effect upon the rate of growth, the relation that they have to the growth of the cell wall is closely bound

up with the fundamental process of growth. There are at least three different hypotheses concerning the nature of the first step in growth:

1. ELASTICITY.—According to the first hypothesis, the growing cell wall must be extended elastically by turgor pressure first; it would at the same time, of course, become thinner. The original thickness is regained either by the incorporation of new particles

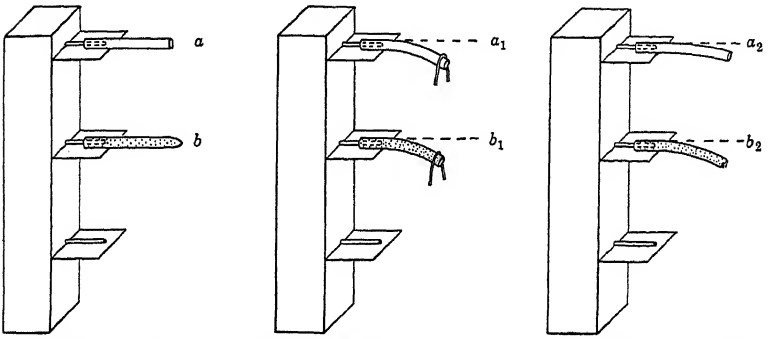


FIG. 35.—Method of determining elastic and plastic extensibility in *Avena* coleoptiles. The coleoptiles were cut away from the seed, the young foliage leaves pulled out, and the resulting hollow cylinders fastened on pins above metal plates. The tips were removed from series *a* one hour before the experiment started, thus depriving them of a growth-hormone supply. The tips were left intact in series *b* until the moment the experiment was begun, hence growth hormone was present (indicated by stippling). Then the tips were removed from series *b* and 250 mg. weights were placed on the ends of coleoptiles in both series. After one hour the curvature was about the same in both series (a_1 and b_1). When the weights were removed, the curvatures decreased. Series *a* without growth hormone retained only 9.3° curvature (a_2). Series *b* with growth hormone retained an angle of 17.3° (b_2). Hence, plastic extensibility was greater in coleoptiles containing growth hormone. (*Adapted after Heyn*, 1931*b*.)

(*intussusception*) or by the laying down of new layers (*apposition*). The force that stretches the cell is, according to this hypothesis, produced by turgor, and the first step in growth is reversible and is not concerned with an increase in substance of the cell wall. A change in the rate of growth, whether general or unilateral, can come about by modification of the extending forces (turgor pressure) or of the elastic extensibility.

2. PLASTICITY.—In the second case, turgor pressure also may be considered the force behind the growth of the cell wall. While the extension considered in the first hypothesis is elastic, *i.e.*, reversible, in this case it is plastic, *i.e.*, not reversible. The first step in growth is not concerned with an increase of cell-wall

substance which can take place later either by intussusception or by apposition. According to this hypothesis, then, a change in the rate of growth can result from changes in either turgor pressure or plastic extensibility.

3. ACTIVE GROWTH.—In the third place, the active growth of the cell wall has been considered as the primary step, a theory propounded by Pfeffer (1904). According to this hypothesis, new particles are laid down between those already present in the wall. Although the turgor pressure is significant in so far as it is necessary to keep the protoplasm in connection with the cell wall, it is not regarded as the source of the energy; rather, this is the result of forces that are active in the secretion of new ingredients for the cell wall. The first step in growth is concerned, therefore, with an increase in the substance of the cell wall; it is not a reversible process and is not influenced by changes in turgor pressure.

Discussion of Hypotheses.—A consideration of the influence of growth substances upon these processes may make it possible to determine the primary step in growth.

1. GROWTH SUBSTANCES AND ELASTIC EXTENSIBILITY.—Heyn (1931*b*) and Söding (1931), at practically the same time, found that a far-reaching parallel exists between growth and elastic extensibility in the oat coleoptile, and Söding made the same observation on flower stalks. Elastic extensibility remains practically constant during the normal growth of the coleoptile, but it decreases when growth is retarded either by decapitation or by complete removal of the coleoptile from the seedling. In decapitated seedlings, the decrease in extensibility may be partly annulled by the addition of growth substance. From this it might be concluded that elastic extension is the primary step in growth and that growth substances make growth possible by increasing the elastic extensibility. Heyn, however, did not interpret his results in this way. The elastic extensibility of excised coleoptiles (removed from the seed and not growing), with growth substance applied, in some of the experiments was greater than the extensibility of excised coleoptiles without applied growth substance. The difference was not great, however, and in both cases the *elastic* extensibility was far less than in growing coleoptiles. The fact that extensibility in nongrowing coleoptiles cannot be increased to any great extent by the addi-

tion of growth substance led Heyn to conclude that changes in elastic extensibility are not the primary cause of growth. Söding (1931, 1932*b*, 1934) came to the same conclusion. He investigated the extent to which curvature, produced by the unilateral application of a tip to a decapitated Avena coleoptile, can be removed by plasmolysis. It was found that while the early part of the growth curvature persists almost entirely, the later part disappears to some extent. Söding concluded from this that the first step in growth is not reversible; hence it cannot be brought about by a difference in elastic extensibility of the cell walls or by changes of turgor pressure or of osmotic concentration. This conclusion is sound as far as can be judged at present.

2. GROWTH SUBSTANCES AND PLASTIC EXTENSIBILITY.—According to Pfeffer's original idea, the limit of elasticity is not reached in turgor extension, thus excluding plastic growth. More recent investigations by Overbeck (1926), Pringsheim (1931), and Heyn (1931*b*) have shown that saturation with water produces an overextension of the cell wall. The question arises whether this plays a part in normal growth. Went (1928*a*) suspected that the effect of growth substances involves an increase in the plastic extensibility of the cell walls; Heyn (1930, 1931*b*, 1934*c*) came to a similar conclusion. The latter determined plastic extensibility according to the method outlined above. It was found that the amount of bending was far greater when the coleoptiles were treated with growth substance before the experiment (see Heyn and van Overbeek, 1931). In coleoptiles which were not growing, plasticity was increased following the addition of growth substance. Similar results were obtained from experiments with hypocotyls of Lupinus (Heyn, 1931*b*, 1934*a*) (Fig. 36). He found, furthermore, that the turgor pressure itself is sufficient to produce an irreversible increase in the surface of the cell wall when the growth substance has increased the plasticity sufficiently. Heyn concluded, therefore, that the primary cause of growth is the plastic expansion of the cell wall. This brings about a decrease in the thickness of the cell wall, which is compensated for by an increase in substance. Gessner (1934) has shown, also, that a close relationship exists between growth and wall extensibility in Helianthus. He concluded that change in wall extensibility is the cause for a change in the rate of growth, not the result of it or an accompanying phenomenon of it.

Although Söding did not state that plastic extensibility cannot assist in growth, he concluded that this is neither the only cause of growth nor the main one. He found that the plasticity of flower stalks is decreased only slightly by topping, although the growth of the stalks suffers a great decrease. Moreover, the differences in the plasticity of individual stalks are considerable. He concluded it improbable "that such a variable and continually changing property as plasticity is the single cause of a regularly occurring process." This implies the assumption that the Avena

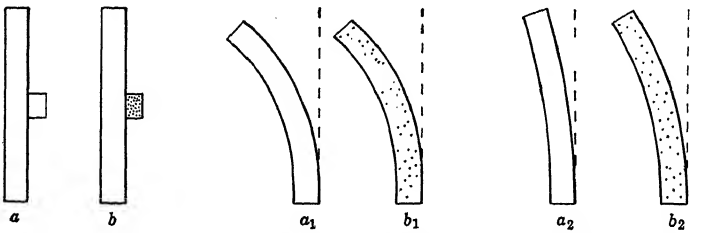


FIG. 36.—Method of determining elastic and plastic extensibility in portions of *Lupinus* hypocotyls. An agar block without growth hormone was applied to a segment from the hypocotyl as shown in *a*. A similar agar block containing growth hormone (indicated by stippling) was applied to another hypocotyl segment, *b*. After 2½ hours the hypocotyl segments were bent by mechanical force to an angle of 45° where they were held for 5 minutes (a_1 and b_1). Upon removal of the force, the hypocotyls returned part way to their original vertical position; the segment treated with growth hormone retained an angle of curvature of 22.9° (b_2), while the control retained an angle of only 12.5° (a_2). Hence, plastic extensibility is nearly twice as great in the hypocotyl treated with growth hormone. (*Adapted after Heyn*, 1934*a*.)

coleoptile behaves in a fashion similar to the inflorescence stalks. In a growth-substance curvature the plasticity of the convex side is greater than that of the concave side, and this difference still remains after the curvature has reached its maximum point, *i.e.*, when no further growth is taking place.

The fact that extensibility in nongrowing coleoptiles is less than in growing coleoptiles is yet to be explained. It is influenced neither by growth substance nor by low temperature (0°C.). Heyn originally concluded that it was brought about by an increase of the cell-wall matter, perhaps by intussusception. Later he showed that the decrease in extensibility is due to a reduction of the extension capacity of the elastically extended outer layers of the wall. Söding considered that the decrease in extensibility may be conditioned by a hardening of the wall and, conversely, that the increase in elastic extensibility of the wall

during growth may be brought about by a softening of the plastic intermicellar substance.

3. GROWTH SUBSTANCES AND INTUSSUSCEPTION.—If plastic extensibility is not the primary cause of growth, as Söding contends, the only possibility remaining is that of intussusception. Pfeffer tried to show that this is of decided importance in the growth of the cell wall. He demonstrated that growth occurs in the root only when the turgor pressure is compensated by an opposing pressure; the growth force must be supplied, therefore, by intussusception.

Söding's hypothesis regarding the growth of the cell wall is this: A viscous intermicellar substance embedded between the micellae can be plastically extended by turgor. This extension, although a part of the growth process, is of only secondary significance. The essential step consists of the addition of new glucose particles from the intermicellar substance to the micellae. At the same time, new intermicellar substance is being formed from the protoplasm, and this increase of matter in the cell wall necessarily is associated with cell elongation. The forces effective in this process are so great that turgor pressure is of secondary significance.

It is clear that we have not yet been successful in determining the primary cause of growth, and it seems best to leave this question open for the present. With regard to the effect of growth substances upon wall growth, there are apparently two possibilities: They influence either plastic extensibility or growth by intussusception.

The first possibility is upheld by Heyn. His view is summarized in the following statement:

> In view of the evidence, it might be expected that the protoplast acts as a mediator in the process of growth-substance activity on the wall. Therefore, the protoplast may be important for the supply of growth substance to a particular portion of the wall, or growth substance may have an effect not directly upon the cell wall but upon the protoplasts which subsequently produce changes in the condition of the wall.

The other possibility—that the growth substance is concerned in growth by intussusception—is upheld by Söding. He assumed

> . . . that the hormone has an effect upon the intermicellar substance directly or indirectly (perhaps through the mediation of the protoplasm)

and stimulates the formation of the structure necessary for growth as well as the whole process of intussusception. Since a greater plasticity of the cell wall (dependent upon the intermicellar substance) occurs in actively growing oat coleoptiles, it may be concluded that the increased plasticity is conditioned physically by this "growth mechanism," *i.e.*, intussusception. It could be considered then that growth is prepared for by the hormone and that increased plasticity of the walls follows. This may be seen from numerous experiments by Heyn (1931*a*, *b*; 1932*a*, *b*). According to this hypothesis, the essential function of the hormone is not to make the wall plastic (Heyn)—if this were the case, only a subordinate process in growth would be influenced (at least in flower stalks)—but its role lies in the regulation of intussusception growth.

Söding presented his views on the mechanism of stretching growth in the following manner:

	Main process	Secondary processes	
Stimulative substance:	Hormone		
Stage of preparation:	Preparation for growth -	Increase in wall extensibility	
		Plasticity	Elastic extensibility
Stage of elongation:	Elongation by intussusception	Plastic turgor extension	Elastic turgor extension

Although a clear picture of the effect of growth substance must await further evidence (see Bonner and Thimann, 1935), several other points may be mentioned in this connection. It is unlikely that growth substance spreads itself out in a monomolecular layer over the growing cell wall and in this way influences growth; the computations of Thimann and Bonner (1933; see also Kögl, 1933, Mitt. III) show that insufficient growth hormone is present to form even a monomolecular film on the growing cell walls; hence, any kind of hormone action through increase of permeability seems improbable. Further work on this problem by Bonner (1934*b*) has shown that cell elongation may not be attended necessarily by a corresponding amount of wall formation. A given amount of elongation may be accompanied by more than the usual amount of wall deposition, as when the tissue is grown in fructose solution; or practically no wall may be laid down, as occurs at 2°C. He concluded that the increase of wall area

probably is not due primarily to active intussusception of new material, at least in the case of the Avena coleoptile. Growth of the wall, according to Bonner (1935), appears to come about by turgor extension of the plastic wall and incorporation of definitely oriented cellulose micellae into the wall.

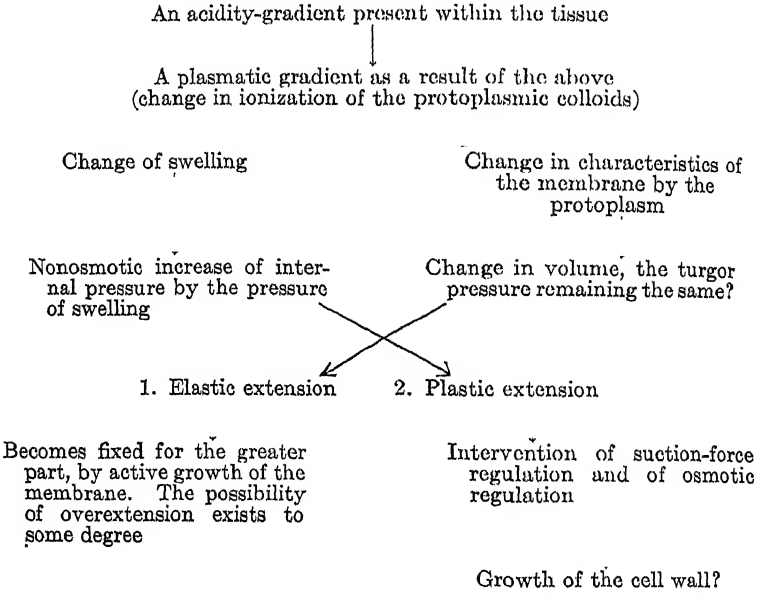
Bonner and Heyn (1935) have tried to determine the influence of growth substance upon the electrical properties of materials composing the cell wall. Suspensions of Avena-coleoptile cell walls, ground and washed in water, were placed in an electrophoresis apparatus, and their movement in an electrical circuit was measured. Apparently the electrical properties of the particles depended chiefly upon constituent proteins. Electrophoresis of material obtained from normal coleoptiles and from coleoptiles that had been decapitated for 2 hours gave no indication of differences in charge. Furthermore, addition of 100 units of 3-indole acetic acid per cubic centimeter of suspension produced no effect upon the charge of the particles. Since neither a direct nor an indirect effect of growth substance upon the charge of the coleoptile cell-wall particles could be demonstrated, it was concluded that the role of growth hormones in promoting cell-wall elongation probably is not exercised through any great modification of the electrical properties of the cell wall.

The theory that growth is promoted through increased plasticity due to the *direct* action of growth substance upon the cell wall seems untenable because of the small amounts of the hormone involved. It seems probable that the growth stimulus is concerned in some way with the processes of the living protoplasm (Bonner, 1933*a*). The many diverse views and the scarcity of sound information concerning the mechanism of growth-hormone activity permit no definite conclusions at the present time.

Acid-growth Reaction and the Growth Hormone.—Another growth theory has been propounded by Strugger (1932, 1933, 1934). He considered as the primary causes of growth all factors that can change the colloidal condition of the protoplasts. It was shown that Helianthus hypocotyls freed of growth substance by decapitation can be influenced to renewed growth by physicochemical treatment of the protoplasts; this was accomplished by immersion of the tissues in suitably buffered solutions, usually by the application of acids. The same effect could be

obtained also in neutral tap water covered with paraffin oil. According to Strugger, the renewal of growth is brought about by acidification; in the case of tap water and paraffin, by acidification resulting from the lack of oxygen. He summarized this view of growth processes in the following scheme:

The primary impetus in growth by elongation is:



Strugger (1933) interprets the significance of growth substances for growth by elongation as follows: "It is clear, therefore, that growth substance obviously does not influence the protoplasm and membrane directly but rather that it regulates the acidity gradients in the course of metabolism and therefore the course and intensity of stretching growth."

Following up the work of Strugger, Bonner (1934*a*) showed that in the Avena coleoptile the effect of acid on growth is not a direct effect upon the physical properties of protoplasm. His study indicates that the "acid-growth reaction" consists primarily in setting free a certain amount of active auxin acid from the inactive salt form of the growth hormone already present in the coleoptile. The growth stimulation by acidification is proportional to the concentration of the free auxin acid. It had been

shown previously that auxin is ineffective at pH 7, more active at pH 6, and about equally active at pH 4 and 5 (Dolk and Thimann, 1932). From these facts it seems quite certain that Strugger's acid-growth reaction is little more than a consequence of the setting free of the auxin acid from its inactive salt.

Inhibition of Growth in Roots.—Up to this point we have observed only the promoting effect of growth substance. It must not be forgotten that the rate of growth in roots is retarded by growth substance. How the same compound can produce the opposite effects in stems and roots has not been explained satisfactorily. Cholodny (1931*a*, *e*) has proposed the hypothesis that growth substance promotes the rate of development of growing cells but shortens the length of their individual life cycles. Elongation in the root lasts only a short time, and the period of growth is shortened still more by growth substance; *i.e.*, if the latter is added, the cells mature quickly without elongating. Growth in length of the root, therefore, is retarded by growth substance. In the stem, on the other hand, the zone of cell stretching is greater, and growth continues for a relatively longer time; the period of growth in stems is shortened (*i.e.*, the rate is accelerated) by the addition of growth substance. There is insufficient evidence to support this hypothesis at the present time.

SUMMARY

Numerous experiments have shown that without growth hormones, growth of the shoots of higher plants cannot take place. Although hormones are not the only important factors concerned in growth, they are essential for the normal enlargement of cells. In the Avena coleoptile, Lupinus hypocotyl, and the foliage leaf of Nicotiana, growth intensity has been shown to be correlated with the differential distribution of hormones. Regions of stems, leaves, etc., where such hormones are present, always appear to undergo greatest growth in one dimension; *i.e.*, growth is *polarized.*

The same substances that are essential for the growth of coleoptiles, foliage leaves, hypocotyls, and stems inhibit the elongation of roots over a wide range of concentration.

The mechanism by which hormones promote growth is not well-understood. There is some evidence that they are used up not as

"building stones" but as activators, influencing in some way the deposition of materials in cell walls. The primary effect of the hormone has been regarded by some investigators as making the cell walls plastic. The stretching that takes place, due to turgor, is accompanied by the incorporation of new wall materials. The result is a permanent increase in size. Other investigators hold that the primary influence of growth hormone is upon the deposition of new wall materials by intussusception and that the forces concerned are so great that turgor pressure is of secondary importance. Whether these or other explanations are valid cannot be decided without more evidence. Growth must be regarded as a function of living protoplasm. The increase in cell-wall boundaries is only one manifestation of the fundamental ability of an organism to build itself out of the materials of its environment.

CHAPTER VII

THE SIGNIFICANCE OF GROWTH SUBSTANCES FOR OTHER PHENOMENA

The significance of hormones for the growth and development of plants appears to be exercised mainly through some effect upon the enlargement of cells. By regulating the increase in cell size, growth substances control the growth of tissues and organs. Recent investigations have extended the role of these substances to include the initiation of roots, the production of tumors, the stimulation of cell divisions in the cambium, and many other important physiological and morphogenetic processes.

Bud Development.—The phenomenon of apical dominance and the inhibition of buds lower down on the shoot axis has been interpreted variously as being due to differential distribution of the food supply, the electrical pattern, or chemical regulators. In recent years, the evidence in favor of some sort of chemical regulation has come to the front.

Since growth substance is a necessary factor for stem elongation, one might consider that the dormancy of resting plant organs, for example, of buds, is caused by a lack of the growth substance. If this hypothesis is correct, then the substance might function to promote growth in dormant tissues. The presence of growth substance in the periderm of dormant potatoes would suggest that other factors must be involved also.

In order to throw more light on this question, the results of some unpublished experiments (by Boysen Jensen) dealing with the influence of growth substance upon resting buds will be discussed. A difficulty in the experimental set-up was encountered in bringing the growth-substance solution into the vicinity of the buds. It was found that Forsythia is suitable for the purpose, since there are diaphragms across the stem at the nodes. If one bores into the stem, the interior can be filled with growth-substance solution. Another method involved removal of the tips from twigs of Salix, Syringa, and Aesculus. The basal end of

each twig was connected with the lower end of a perpendicularly suspended funnel 20 cm. long. When this was filled with growth-substance solution, the fluid flowed slowly through the twig and exuded from the apical-cut surface. These experiments were carried out with different concentrations of growth substance during the winter when the buds were still in a resting condition. In no case was it possible to observe any "forcing" effect as a result of the treatment.

Various investigators have studied the retarding effect which the axis of the shoot has upon the development of the axillary buds. According to Snow (1925*a*, 1929*a*, *b*, 1931*a*, *b*, 1932*a*), this is caused by a specific retarding substance. Thimann and Skoog (1933) and Skoog and Thimann (1934) have reported that this substance may be identical with growth substance. When the terminal buds were removed from seedlings of *Vicia Faba*, the lateral buds developed rapidly. This activity could be retarded by placing agar blocks with relatively large amounts of auxin upon the cut surfaces. These investigators concluded, therefore, that the growth substance formed by the terminal bud in normal, nondecapitated plants is the retarding factor in the development of lateral buds. When these start to develop, growth substance is formed, and this has its effect upon their further development. If the hypothesis of Thimann and Skoog is correct, it follows that the growth substance formed by buds can both inhibit and promote their development.

Uhrová (1934) found that a substance diffusible into agar or gelatin was present in the leaves of *Bryophyllum crenatum* and inhibited lateral bud development. Hormone from Avena coleoptiles, diastase, and saliva, as well as acids had the same effect.

Czaja (1934) and Söding (1935*a*) have demonstrated growth substance in the developing buds of many woody plants, and Avery (1935) has found it in the young growing leaves of Nicotiana. The relationship between the processes involved in bud development and the role of growth substance has not been discovered. Further studies by Hitchcock (1935*b*) have shown that lateral bud inhibition can be brought about by the application of indole-acetic and indole-propionic acids or ethylene and propylene gases to decapitated tobacco plants. Bauguess (1935) has found inhibition effects with β-3-indole pyruvic, β-3-indole-oximinopropionic, and β-3-indole acrylic acids.

Tumor Formation.—Growth substances not only function by regulating normal growth but also, under certain conditions, bring about abnormal swellings and intumescences by hypertrophy of the tissues of stems and leaves. Cholodny (1931*a*, *e*) placed coleoptile tips laterally upon the root tips of maize. He found that tumor formation occurred and that the zone of elongation of the swollen roots was significantly shortened. Anatomical investigation revealed considerable enlargement of the cortex. The number of cell layers was not changed, but radial and tangential dimensions of the single cells were remarkably increased, and they were less susceptible to dyes than normal root cells.

According to Loeb (1924), callus formation occurs only at the basal, never at the apical, end of a stem of Bryophyllum. A small piece of stem without leaves forms little or no callus, while a piece of stem of the same mass with a leaf attached to it forms considerable callus.

Callus formation has been found to result from the application of lanolin paste containing an extract obtained from orchid pollinia (or human urine) to the internodes of Tradescantia and Coleus (Laibach, Mai, and Müller, 1934). The active agent, supposedly promoting cell division, was termed *meristine*. Since it was found to be soluble in ether and water and is thermostable, it was considered identical with auxin. Laibach and Fischnich (1935*a*) devised a quantitative method of testing the callus-forming action of 3-indole acetic acid paste (and other substances) which brings about an increased rate of cell division in *Vicia Faba* epicotyls (Fig. 18*A*). Laibach (1935) reported that urine, pollinia, and corn-flower pastes, when applied to decapitated *Vicia Faba* epicotyls and to Coleus stems, all brought about callus formation in 3 days.

Czaja (1935*c*) has criticized Laibach's conclusion that a cell-division effect has been demonstrated for growth substance in callus formation. He found that if the growth substance is applied on the side of the axis so that it moves inward in opposition to the normal stream of the substance, swelling results below the place of its application. These swellings arise by the transverse stretching of the cells. Complete disorganization of the normal polarity leading to cambial activity and the subsequent formation of masses of tracheids on the apical end of the organ

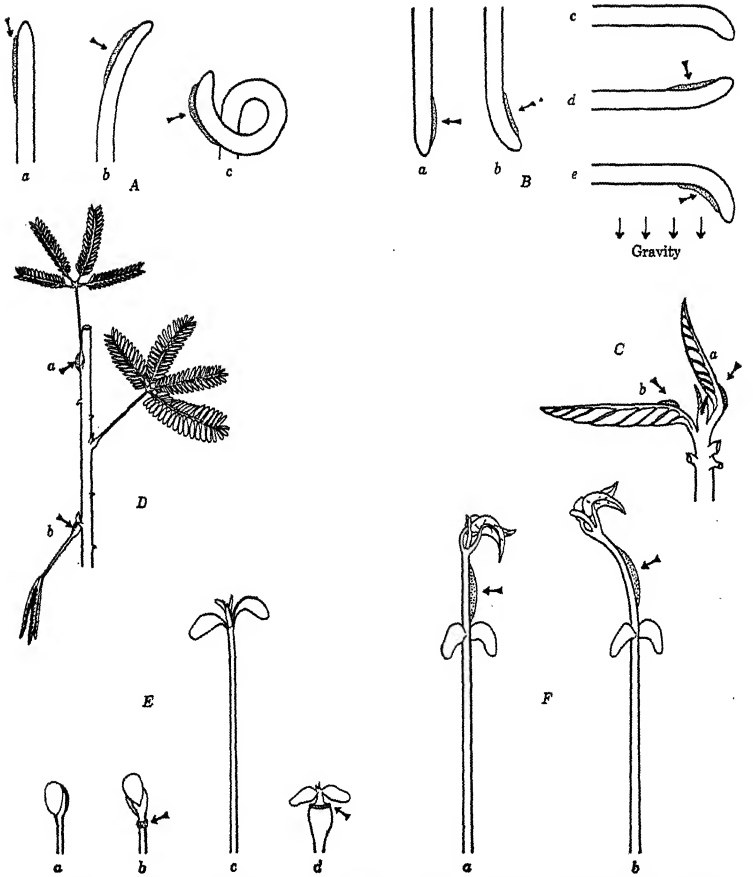


FIG. 37.—Various phenomena brought about by the application of growth hormone to plants. The paste containing the hormone is indicated by stippling. *A*, unilateral application of 3-indole acetic acid paste, as shown in *a*, induces curvature in the *Avena* coleoptile (*b* and *c*). (*After Laibach*, 1933*b*, 1935.) *B*, growth curvature in roots may be regulated by the unilateral application of growth hormone: *a*, when applied to a vertical root, lateral bending occurs, as shown in *b*; *c*, primary roots in the horizontal position normally curve downward, but growth hormone applied on the upper side causes negative geotropic curvature, *d*; applied on the lower side of a horizontal root it promotes positive geotropic curvature, *e*. (*After Koch*, 1934.) *C*, young leaves of *Nicotiana* and other plants exhibit hyponasty, *a*, and epinasty, *b*, as a result of the addition of 3-indole acetic acid paste. (*After Avery*, 1935.) *D*, *Mimosa* petioles bend upward, *a*, or downward, *b*, upon application of 0.01 per cent 3-indole acetic acid paste to the lower or upper sides of the primary pulvini. (*Burkholder and Pratt*, 1936.) *E*, application of growth-hormone paste around the hypocotyl of a *Helianthus* seedling nullifies polarized growth in the long axis: *a*, normal seedling; *b*, with ring of hormone paste; *c*, untreated seedling; *d*, treated seedling, after 7 days. (*After Czaja*, 1935*a*.) *F*, if growth hormone is applied unilaterally to a young stem, as shown in *a*, bending occurs away from the side of application, *b*. (*After Zimmerman and Wilcoxon*, 1935.)

were thought to arise as secondarily induced phenomena following the artificial addition of substances causing cell enlargement. In another paper, Czaja (1935*a*) reported the results of further investigations dealing with the effects of growth substance over a considerable range of concentration when applied to Helianthus, Avena, and other plants (Fig. 37*E*). Additional evidence was obtained for the way in which cells increase their

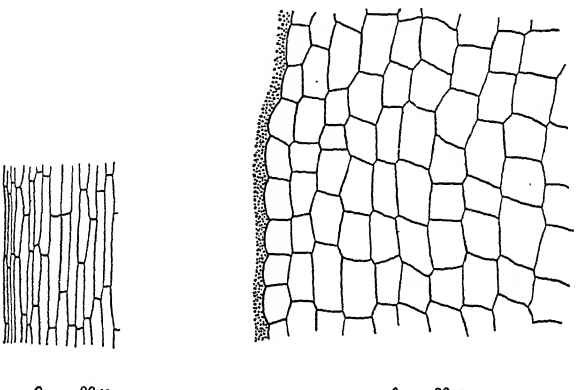


FIG. 38.—Longitudinal sections of *Helianthus* hypocotyls, showing effects of the application of 3-indole acetic acid on polarized growth. *A*, the normal cortex is comprised of cells markedly elongated in the direction of the long axis of the organ. *B*, cells in a hypocotyl treated with growth-hormone paste become nearly isodiametric, and have a much greater volume than those in the normal hypocotyl. (*After Czaja*, 1935*a*.)

dimensions under the influence of growth-substance supply. The lateral application of relatively high concentrations brought about retardations of growth in length and increase in thickness of roots, stems, etc. (Fig. 38). It was concluded that the direction of growth by cell enlargement is controlled by the direction of transport of growth substance.

Recently LaRue (1935) has investigated the role of the auxins in the development of intumescences on poplar leaves. The injection of heteroauxin into twigs or its application in lanolin directly upon the leaves brought about proliferation of tissue. Cell outgrowths from the mesophyll of *Mitchella repens* were produced by the feces of insect larvae or applied droplets of 0.0005 per cent 3-indole acetic acid.

Stomatal Movement.—In an attempt to find out whether growth substance influences the production of starch in the guard cells and therefore possibly affects the opening of the stomata, Boysen Jensen removed the petioles of leaves of Sinapis and Sambucus and placed them in water and in growth-substance solutions of different concentrations. Although the growth-substance solution was taken up by the leaves in abundant amounts, no effect upon the degree of opening of the stomata could be distinguished, nor could any increase or decrease of starch in the guard cells be observed.

Respiration.—Boysen Jensen and Nielsen (1925) published some experiments dealing with the effect of decapitation on the intensity of respiration in Avena coleoptiles. It was found to be practically the same in decapitated and nondecapitated coleoptiles, and no effect of growth substance upon respiration could be shown. Nielsen and Hartelius (1932) also found that rhizopin was without effect in this respect. According to the more recent experiments of Bonner (1933*a*, *b*), however, growth substance may influence the intensity of respiration of the Avena coleoptile. Coleoptile cylinders 3 mm. long were placed in growth-substance solution, and the intensity of respiration was determined. In the lower concentrations the rate was promoted about 27 per cent; and at higher concentrations there was a retardation. When the growth substance was inactivated by treatment with peroxide, no promoting effect could be observed.

Bünning (1934*b*) concluded from his observations on Aspergillus that the assimilation of nitrates was increased by the promoting effect of growth substance on respiration. The effect was brought about by moving the pH in an alkaline direction, which increased conidia formation.

Cambial Activity.—The stimulating influence of foliage leaves upon cambial activity has been suggested by a number of workers (Coster, 1927; Thoday, 1933). Kastens (1924) had suggested that the stimulus for cambial activity may be a hormone. The observations of Snow (1933) made it seem probable that the stimulus contributed by developing buds might be some chemical substance in the nature of a hormone. Snow and LeFanu (1935*a*) obtained indications of increased cell division in the cambium of young Helianthus plants which had been decapitated and treated with urine extracts. In more detailed experiments these same

workers (Snow and LeFanu, 1935*b*; Snow, 1935*a*, *b*) used purified auxin *a* and 3-indole acetic acid in concentrations of 1 or 2 p.p.m. When aqueous solutions of these substances were applied to the upper ends of decapitated Helianthus seedlings, cambial division was stimulated.

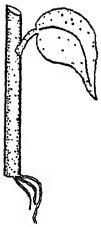
The demonstrated presence of growth substances in sprouting buds and young leaves, and the effectiveness of these substances in promoting cambial activity, lead to the conclusion that the earlier suggestions of a hormone stimulus passing from the leaves to the cambium in the stem is probably true. The precise way in which the auxins bring about increases in both cell size and number in different plants under different circumstances is not yet clear.

Nastic Movements.—The great volume of evidence concerning the role of growth substance in tropic curvatures suggests strongly that some similar mechanism may be involved also in nastic responses. The differential growth of bilaterally symmetrical organs, in response to stimulation by light, temperature, electricity, touch, gases, etc., has been studied in detail (Hennings, 1930; Zimmermann, 1931, 1932; Zeltner, 1932; Schmitz, 1934; etc.), but only recently has the growth-substance explanation actually been tried out experimentally in this connection. Avery (1935) showed that epinasty or hyponasty could be produced readily in Nicotiana by applying a small amount of growth substance in lanolin to the adaxial or abaxial surface of the petiole (Fig. 37*C*). The well-known movements of Mimosa have been found to be remarkably influenced also by the application of 3-indole acetic acid to the pulvini (Fig. 37*D*) (Burkholder and Pratt, 1936). Nastic movements of Coleus leaves following treatment with 3-indole acetic acid (0.5 per cent in paste or solution) have been studied in detail by Fischnich (1935). The amount and duration of response varied directly with the concentration of the applied substance. Hitchcock (1935*a*, *b*) has demonstrated recently that a number of different substances, when applied to leaves of tobacco and tomato, are capable of bringing about epinastic movements. The decreasing order of effectiveness for a series of compounds producing epinasty was as follows: naphthalene acetic and indole acetic acids, indole butyric and indole propionic acids, phenylacetic, phenylpropionic, and phenylacrylic acids. The production of leaf epinasty by ethylene, acetylene, propylene,

and carbon monoxide gases (Crocker, Hitchcock, and Zimmerman, 1935) and by α-naphthalene acetic acid, β-naphthalene acetic acid, acenaphthyl-5-acetic acid, fluorene acetic acid, anthracene acetic acid, and α-naphthyl acetonitrile (Zimmerman and Wilcoxon, 1935) is of great interest. Bauguess (1935) has reported similar epinastic effects with other organic acids. Further investigations are needed to discover the mechanisms that lead to these manifestations.

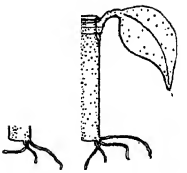
Root Formation.—The influence of many kinds of substances upon the initiation and growth of roots has been studied by numerous workers, but only in comparatively recent years has it become apparent that the old hypothesis of root-forming substances, proposed by Sachs (1882*a*), may possibly have some basis in fact (see also Morgan, 1903). Loeb (1916, 1917, 1924) performed some very instructive experiments which led to the observation that a stem segment of Bryophyllum forms roots and bends geotropically more readily when a vigorous leaf remains attached to the stem (Fig. 1, Loeb). As a result of his observations, he wrote: "All these facts suggest a close association if not identity between the root-forming substances and the substances (or hormones) causing geotropic curvatures." The significance of this statement was not realized until the more recent developments in the field of plant hormones.

The work of van der Lek (1925, 1934), showing that the presence of leaves or buds promotes the formation of roots at the morphological base of a cutting (Fig. 39), led to further investigations of the possible role of hormones in the initiation of roots. Following this line of attack, F. W. Went (1929) found that a nonspecific, heat-resisting substance could be extracted from leaves and germinating barley which, when applied to cuttings, promoted the development of new roots (Fig. 39). F. A. F. C. Went (1930) investigated the root-forming substance in *Bryophyllum calycinum.* The function of root-forming substances was studied further in Impatiens and Acalypha by Bouillenne and Went (1933) (see Bouillenne, 1933). Laibach, Müller, and Schäfer (1934) demonstrated the formation of roots by urine extracts applied to internodes of Tradescantia, Helianthus, and Ligustrum. Laibach (1935) and Fischnich (1935) obtained similar results with 3-indole acetic acid on Coleus (Fig. 39). Went (1934*a*) named the substance that stimulates root formation *rhizocaline* and

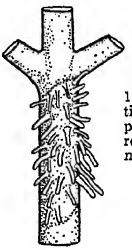


Van der Lek, 1925. The presence of a developing bud or young leaf promotes root formation in a woody cutting.

Went, 1929. A substance stimulating root production is contributed to a stem by a grafted leaf, *c*, or agar containing boiled diastase solution, *b*; the control stem, *a*, remains without roots.

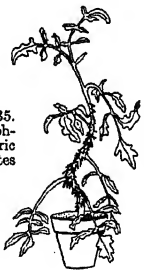


Went, 1934*b*. *Thimann and Koepfli*, 1935. A quantitative test for substances stimulating root production may be made by immersing the apex of a split stem in solution for 15 hours. After two weeks' growth in water, the number of roots gives a measure of the concentration of the substance. (See Fig. 40 for further details.) With this method, it was established that rhizopin, auxins, and "rhizocaline" are identical.



Laibach, Müller, and Schäfer, 1934. *Laibach*, 1935. Application of 3-indole acetic acid paste to a Coleus stem causes roots to be produced in great numbers.

Zimmerman and Wilcoxon, 1935. Injection of indole acetic acid, naphthalene acetic acid, indole butyric acid, etc., into plant stems stimulates root production.



Hitchcock and Zimmerman, 1935. Addition of 3-indole acetic acid or any one of several other synthetic growth substances to the soil stimulates root production on aerial parts of Lycopersicon and Nicotiana.

Zimmerman, Crocker, and Hitchcock, 1933. Treatment of *Tagetes* plants with 1.0 per cent carbon monoxide mixed with air for 2 to 10 days stimulated the production of adventitious roots on the stem.

Zimmerman and Hitchcock, 1933. *Tagetes; a*, roots were produced six days after a 72-hour treatment with 0.25 per cent acetylene; *b*, a second treatment with gas caused a change in the direction of growth and the production of root hairs.

FIG. 39.—Rooting phenomena.

worked out the proportionality existing between its concentration and the number of roots formed in pea seedlings (Fig. 39). The details of the method may be obtained from Fig. 40. Thimann and Went (1934) found that the active substance was present in large quantities in the crude auxin extracts obtained from Rhizopus and also from urine. Then it was found that the

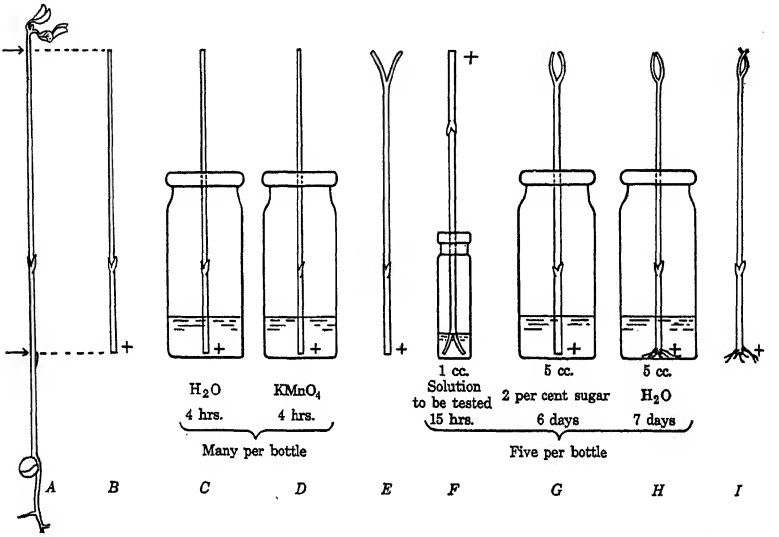


FIG. 40.—A method of testing for the presence of substances stimulating the production of roots. The number of roots formed indicates the approximate effectiveness of the solution. The test is made in a darkroom at 25°C. and at a relative humidity of 60 to 70 per cent. *A*, etiolated pea seedling 7 days old, cut above first scale leaf and again just below the tip, gives test plant *B*. Basal end of shoot marked +. After standing in water 4 hours, *C*, and in potassium permanganate 4 hours, *D*, the shoot, *E*, is split longitudinally at the apical end with a sharp razor blade, and the base rinsed with water. It is immersed 15 hours in the solution to be tested, then rinsed with water and placed in sugar solution where it is allowed to stand for 6 days, *G*. Next it is removed from the sugar solution, rinsed with water and placed in water for 7 days, *H*. Two weeks after stage *A*, the test plant *I* is examined for the number of roots present. (*After Went*, 1934*b*.)

pure auxins prepared by Kögl and coworkers were effective in root formation (Thimann and Went, 1934) as well as in growth promotion. Thimann and Koepfli (1935) showed that 3-indole acetic acid stimulates root production; hence substances causing cell elongation are also effective in root formation. The role of pollen and urine extracts in root formation has been studied further by Müller (1935) and by Laibach and Fischnich (1935*b*).

The latter workers have shown that in horizontally placed stems there is a displacement of root-forming substances from the upper to the lower side just as is the case for the cell-elongation substances. Initiation of roots is brought about in a region where longitudinal transport of the substance is prevented, *e.g.*, by a wound. The synthesis of root-forming substances in leaves through the action of orange-red light and their polar transport

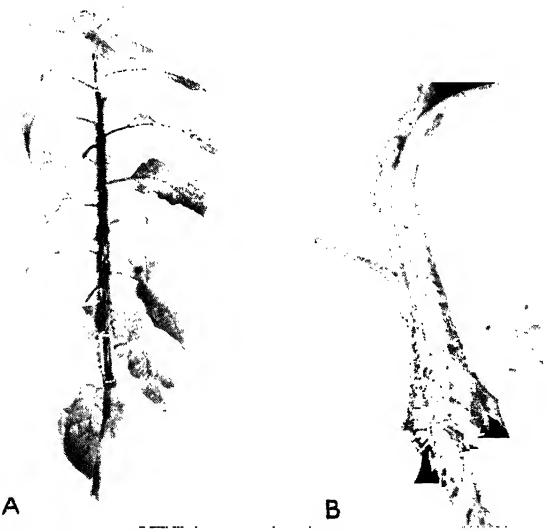


FIG. 41.—Rooting effects obtained by applying synthetic substances to plants. *A*, *Nicotiana*. An overhanging slit portion of the stem is immersed in 0.05 per cent indole butyric acid. The acid travels upward in the transpiration stream and causes adventitious roots to be produced along the stem. *B*, *Lycopersicon*, adventitious roots on stem 14 days after treatment with 2.0 per cent 3-indole acetic acid in lanolin. (*From Zimmerman and Wilcoxon*, 1935.)

have been pointed out recently by Went (1935*b*). Attention should be called to the fact that the same substances that cause formation of roots also inhibit their growth in length.

That the auxins are not unique in their ability to produce new roots has been shown by a series of contributions from the Boyce Thompson Institute. The initiation and stimulation of adventitious roots by treatment with appropriate doses of ethylene, acetylene, propylene, and carbon monoxide gases have been demonstrated in some 15 species and varieties of plants (Fig. 39) (Zimmerman and Hitchcock, 1933; Zimmerman, Crocker, and

Hitchcock, 1933*a*, *b*). The effects of 16 different "growth substances" applied to stems and leaves either in the form of paste or

FIG. 42.—Rooting and nastic responses obtained by applying synthetic substances to *Lycopersicon*. *A*, left: control plant, decapitated; right: plant with surface of cut stem treated with 1.0 per cent α-naphthalene acetic acid; photographed after 8 days. *B*, left— control plant; right— plant after injections of 0.01 per cent indole butyric acid, photographed after 14 days. Note induced epinasty of leaves and adventitious roots. (*From Zimmerman and Wilcoxon*, 1935.)

in solutions have been considered in relation to the local initiation of adventitious roots on these organs (Figs. 41 and 42) (Zimmerman and Wilcoxon, 1935). α-Naphthalene acetic acid and

indole butyric acid were the most effective substances for the stimulation of new roots. The mechanism of action of these different compounds is not known.

Miscellaneous.—Since the discovery of growth substances in plants, the effect of these substances when externally applied to plant organs has revealed a wide variety of interesting phenomena.

The influence of growth substance upon regeneration and the forming of wound tissue has been studied by Mrkos (1933). The fluid obtained from cultures of *Rhizopus suinus*, excised Zea coleoptile tips, and portions of Bryophyllum leaves was found to promote cell division in the wounded mesophyll of Bryophyllum leaves. These studies seem to support the hypothesis of "wound hormones" proposed by Haberlandt (1921, 1922). Mai (1934) investigated the significance of growth substance for the elongation of petioles and reported a prolonged period of petiole growth due to the application of pollen paste to Coleus, Acer, Viburnum, and other plants. Laibach (1934) demonstrated the curling of leaves by applying growth-substance paste to young leaves. LaRue (1935) found that when the blades were removed from the petioles of Coleus, abscission soon followed. Applications of agar blocks or lanolin containing exudate of leaves or pollen of *Populus grandidentata*, 2 per cent urine, or 0.0005 per cent heteroauxin caused the petioles to remain on the stems from 35 to 141 per cent longer than in the controls.

SUMMARY

It has been shown in Chap. VI that hormones regulate the normal growth of plants by promoting cell enlargement in expanding organs. Many other effects have been attributed to these growth substances. One of the most interesting of these is concerned with the phenomenon of chemical correlation within the plant body. Growth substance apparently is formed in expanding buds and rapidly growing leaves whence it moves into other regions of the plant, exercising some degree of control over the behavior of potential centers of growth. It has been shown further that auxin *a* and heteroauxin may stimulate cell division in the cambium; hence it is probable that cambial activity in dicotyledons is stimulated by the growth hormones supplied from growing leaves and buds.

Under the proper circumstances, growth substance may bring about the formation of tumors, the building of callus tissue, and the initiation of new roots. The substances present in the coleoptiles of maize, orchid pollen, urine, and also pure heteroauxin have been shown to bring about an increase in the bulk of the tissues near the site of their application in both roots and stems. Such hypertrophy may be brought about either through an increase in the number of cells or by a shift in the direction of growth. Proliferation of cells has been demonstrated, too, by the application of heteroauxin to mesophyll tissue.

The role of the auxins in the neoformation of roots has been demonstrated in numerous instances, as has the fact that these same substances also inhibit the growth in length of roots. That the auxins are not unique in their ability to produce new roots has been shown by the effectiveness of many other substances in this respect, such as, α-naphthalene acetic, indole butyric acid, etc. The mechanism of action of these different compounds, whether direct or through some influence upon the auxins or in still other ways, is not known.

The differential growth on the two sides of bilaterally symmetrical organs leading to nastic movements has been shown to be influenced by the local application of auxins and divers other substances. For example, heteroauxin caused marked nastic responses in the leaves of such plants as Nicotiana, Mimosa, etc., and a number of other substances have been shown to bring about epinasty in Nicotiana and Lycopersicon. Prolongation of the growth period and prevention of petiole abscission by auxins, also, have been demonstrated in several species. Future investigations may provide an explanation of the fundamental mechanism of growth-substance activity in connection with these diverse manifestations of hormone-controlled growth.

CHAPTER VIII

THE SIGNIFICANCE OF GROWTH SUBSTANCES FOR PHOTOTROPISM

When illuminated from one side, plant shoots usually turn toward the light. This response is brought about by differential growth, in such a way that curvature results. Though the direction of movement is usually positive, there are some instances in which organs turn away from the light; in such cases, they are said to be negatively phototropic. Inquiries into the nature of phototropism led to the discovery of substances that regulate growth in plants; continued studies on this subject have helped elucidate the mechanism of tropic responses.

GENERAL DISCUSSION OF PHOTOTROPISM WITH SPECIAL REFERENCE TO THE AVENA COLEOPTILE

The bending responses to light have been studied in many kinds of plants, but the favorite object for investigations dealing with the mechanism of phototropic curvature is the coleoptile of *Avena sativa*. Certain distinct advantages in its use arise from the fact that seeds from pure lines of the species can be obtained practically everywhere, the plants can be grown easily in any laboratory, and reproducible results can be obtained under controlled environmental conditions.

Stimulation and Response. *The Light Gradient.*—When an Avena coleoptile is illuminated unilaterally, light is absorbed by the tissues, and a descending gradient of light intensity across the organ results. Under these circumstances, that part of the coleoptile nearest the source of light naturally receives a greater amount of light than the portion on the shaded side of the organ. This gradient of the light stimulus, which is of considerable importance for the theory of phototropism, has been measured with great care by several investigators.

Lundegardh (1922) tried to determine the magnitude of the light decrease in unilaterally illuminated oat seedlings by employing photometric methods. It was found that the light in the

coleoptile tip is diminished by only one-tenth when the back side is not shaded by the primary leaf. In other words, the shaded side receives nine-tenths of the light which falls upon the side nearest the source of light. In the basal region, however, the far side obtains only one-twentieth to one-fiftieth the amount of the light received by the front side. Van Dillewijn (1927*a*) found, in rather good agreement with these measurements, that the back side receives about one-thirtieth as much light as does the front.

Nuernbergk (1927) since has found lower absorption values in unilaterally illuminated coleoptiles than were indicated by earlier workers. When the broad side was illuminated, the light was decreased to about one-seventh in the basal region; when the narrow side was illuminated, the light value on the far side was reduced to one-tenth of the incident amount. The decrease in intensity in the apical zones depended somewhat upon whether or not the primary leaf was within the coleoptile. With the leaf present, the front side received 4 to 8 times as much light as the back. With the leaf not acting as a screen, the front side received 1.3 to 1.5 times as much light as the back when illuminated on the broad side and 2.6 to 3 times as much when illuminated on the narrow side. This difference in the absorption values for the narrow and wide dimensions of the organ is probably due to the difference in thickness of the tissues through which the light passes. As shown in Fig. 21, the walls of the coleoptile are much thicker in the narrow portions containing the bundles than in the other parts of the organ.

Bergann (1930) also determined the decrease of light in the Avena coleoptile by microphotometric measurements. It was found that the blue rays passing through the coleoptile tip from the broad side were 2.2 times and from the narrow side 3.0 times more intense on the front than on the back side. In the basal portion of the coleoptile the intensity was 33 to 37 times greater on the exposed side. However, the stimulating effect of light arises mainly by its action upon the apical portion of the tip.

It should be mentioned that the height of the primary leaf in the hollow cylinder of the coleoptile has in itself no significance for the course of phototropic curvature, although the presence of the leaf, acting as a light screen, has a decided influence upon the light gradient across the coleoptile. Du Buy (1934) studied the

light gradient and phototropic curvature in Avena coleoptiles which were filled with water or with air. Curvatures were less in the water-filled coleoptiles owing to a smaller light gradient.

Distribution of Sensitivity to Light.—Early studies upon the question of relative phototropic sensitivity of different zones of the oat coleoptile established the fact that not all regions are equally sensitive to light (Darwin, 1899; Rothert, 1892, 1894). Rothert concluded from the results of his studies that "the apical region of high phototropic sensitivity is not longer than 3 mm., and only in the extreme 1 to 1.5 mm. portion of the tip is the sensitivity to light particularly great."

Since the studies by Rothert, this same question has been investigated with improved technique. Sierp and Seybold (1926) used adjustable screens in order to partially darken an exactly determinable portion of the tip. The presentation time was then determined when 0.25, 0.50, 0.75, to 2 mm. portions of the tip were darkened. From the figures obtained in this manner, a curve was constructed which showed an increase in presentation time with an increase in the length of the darkened apical region. Sierp and Seybold stated their results as follows:

> In conclusion it can be said that the sensitivity to light in the *Avena sativa* coleoptile is greatest in the ¼ mm. at the tip, from which point downward it decreases rapidly. In the zone of about ¼ to ½ mm. lying directly below the tip region, the sensitivity is only $\frac{1}{40}$ of that at the extreme tip. At a distance of 2 mm. from the tip, sensitivity decreases to 1/36,000 of that of the uppermost region; and from this point on to the bottom, it remains about constant.

At about this time, Lange (1927) studied the distribution of sensitivity to light in the coleoptile tip. He thought that the method of darkening used by Sierp and Seybold could cause half-shadow formations which would impair the accuracy of the measurements. To avoid this, he illuminated each transverse zone to be investigated by means of a slit, the width of which could be diminished to 50 microns. The light value was determined as the product of the intensity of light × time of illumination × area of the illuminated surface. The threshold values necessary to elicit phototropic response were determined for different zones of the coleoptile. From the formula:

$$\text{Sensitivity to light} = \frac{k}{\text{threshold light value}},$$

where k can be any desired constant, the sensitivity to light may be computed. A comparison of the experiments of Lange and of Sierp and Seybold is given in Table 4, where the values obtained

TABLE 4.—RELATIVE SENSITIVITY TO LIGHT IN DIFFERENT ZONES OF THE AVENA COLEOPTILE

Zone, millimeters from apex	Light sensitivity, as calculated from the experiments of	
	Sierp and Seybold	Lange
0 – ½	33,948	38,870
½–1	564	2,870
1 –1½	34.7	253
1½–2	8.42–24.2	24.7

by the latter authors have been recomputed on the basis of Lange's formula. Even though the two series of numbers deviate conconsiderably from each other in the second and third half-millimeter zones, the results are essentially in agreement.

Since Lange worked with zones as narrow as 50 microns, he has given us very exact information concerning the distribution of sensitivity to light in the extreme tip region. It is clear that the uppermost zone, which is only about the width of a single cell, is the point most sensitive to light (see Table 5). Phototropic

TABLE 5.—RELATIVE SENSITIVITY TO LIGHT IN THE UPPER HALF MILLIMETER OF THE TIP OF THE AVENA COLEOPTILE

Zones, Microns	Sensitivity
0– 50	7,600
50–100	6,422
100–200	5,665
200–300	4,106
300–400	3,046

perception diminishes rapidly when the tip is removed (Koch, 1934). The reduction in sensitivity becomes more marked with the removal of tip pieces up to 1 mm. in length, just as has been observed by darkening different zones. With still further decapitation, the increase is much more than that found by the darkening method. After some time has elapsed following decapitation, a "physiological tip" is regenerated; at the same time, phototropic sensitivity is increased, and the ability to react

to light stimuli is restored (Dolk, 1926; Reinders, 1934). It should be mentioned that general illumination of coleoptiles during the growth period lowers their sensitivity to subsequent unilateral lighting (Filzer, 1930).

Interesting data on the differential response of the tip and base of the Avena coleoptile to varied intensity and controlled wave lengths of radiation have been obtained recently by Haig (1935). The extreme tip portions (1.5 mm.) of some plants and the subapical regions of other plants were exposed to white light for short periods, and the reaction time was measured for positive phototropic curvature. The speed of the initial reaction-time process was found to be proportional to the logarithm of the light intensity up to about 1,000 meter-candle seconds, above which there was a marked decrease in the rate of response. The reaction-time curves for the tip and for the rest of the coleoptile yielded separate loci suggestive of two distinct photoreceptive systems. With white light, blue-green, and minus-blue regions of the spectrum, the tip was phototropically more sensitive than was the base. These results support the earlier work of Went (1926), who reported a distinct difference in the light sensitivity of the tip and base of the Avena coleoptile.

The phototropic sensitivity of the Avena coleoptile to different regions of the spectrum has been determined in recent years by several investigators (Bachmann and Bergann, 1930; Johnston, 1934). It seems to be well-established that the shorter wave lengths of visible light are most active in causing phototropic curvature, while the longer wave lengths in the red region are practically inactive (Fig. 43). The relative effectiveness in the region of greatest sensitivity (*i.e.*, in blue light) is represented by a bimodal curve with maxima at about 4,400 and 4,800Å. (Johnston, 1934). As a matter of interest it may be mentioned here that a similar bimodal curve with maxima in the same regions (Fig. 44) has been found for the light inhibition of germinating lettuce seed (Flint and McAlister, 1935). The phenomenon of differential spectral sensitivity would appear to be of considerable significance in connection with an analysis of phototropism and growth.

Conduction of the Stimulus.—The early studies (Rothert, Fitting, Boysen Jensen) on the conduction of the phototropic stimulus in the Avena coleoptile were described in the first chap-

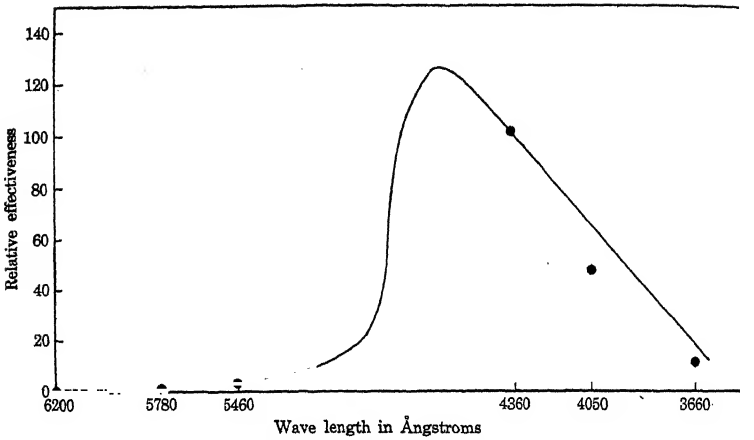


FIG. 43.—Spectrum sensitivity curve of phototropic response (solid line) and protoplasmic streaming (points) in the *Avena* coleoptile. The agreement between the phototropic response and the effect of light on retarding protoplasmic streaming suggest an interrelationship. (*After Bottelier*, 1934.) (*Curve of phototropic response from Blaauw*, 1909.)

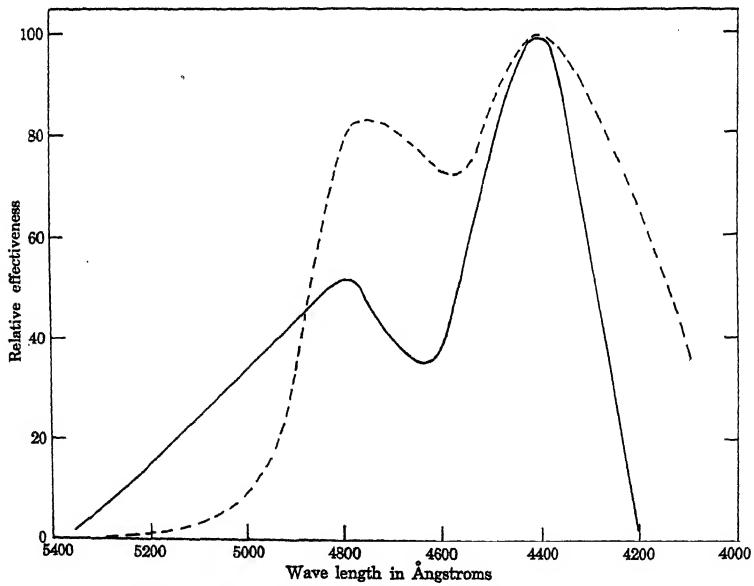


FIG. 44.—Curve of inhibition of *Lactuca* seed germination (solid line) in the violet-green region compared with the curve of phototropic response of *Avena* coleoptiles (broken line). (*After Flint and McAlister*, 1935.) (*Curve of phototropic response from Johnston*, 1934.)

ter. In these experiments, transverse incisions were made upon the front (lighted) or back (shaded) side of the coleoptile a few millimeters below the tip. In some cases, mica plates were inserted in the cuts. Then the tip was unilaterally illuminated above the incision, and it was determined whether the cut had interfered with the conduction of the stimulus to the growing portions below. Boysen Jensen's experiments showed that the stimulus is conducted on the back (shaded) side of the Avena coleoptile. Later, Purdy (1921) carried out quantitative studies dealing with the localization of the stimulus conduction. In order to eliminate the stimulus resulting from making the incision, the experimental plants were used 24 hours after the operation, when they were again entirely straight. Pieces of mica were then inserted into the incisions, and the tips of the plants were unilaterally illuminated, with the pieces of mica facing either toward the front or the back. The inconsequential curvature, which was caused in some cases by the insertion of the mica pieces, was measured and taken into account. When the incision was on the front side, a phototropic curvature resulted with a *d* value (see Chap. II) of 0.61 mm.; in other cases, of 0.10 mm. (Fig. 45). Purdy concluded from her experiments "that the strongest tendency is for transmission of the stimulus to take place in a longitudinal direction, mainly localized in the side of the coleoptile farthest from light."

The importance of the vascular bundles in conduction of the phototropic stimulus from the tip downward has been indicated in the experiments of Baffet *et al.* (1933) with glass plates inserted transversely in the Avena coleoptile.

Beyer (1928*a*) tried to show that conduction of the phototropic stimulus can take place upon the front as well as upon the back side. In his experiments, the basal portion of the plants were darkened with sand. According to Cholodny (1929*a*), sand is translucent and is not sufficient to protect the basal portion of the coleoptile entirely from light (see also Reinhard and Bro, 1933).

Conduction of a stimulus upon the front side of the coleoptile, although a weak one, has been repeatedly observed, first by Purdy and later by others. From the standpoint of the growth-substance explanation, such a phenomenon might be expected, but the stimulus transmission that takes place upon the front side is of an entirely different nature from that occurring on the back

the convex and concave sides resulting from unilateral illumination with 80 meter-candle seconds, after different reaction times. In this experiment, the front and back sides of the unilaterally illuminated coleoptile are exposed to the same two light intensities as the two coleoptiles mentioned, owing to the light gradient through it. This should yield information of value as to whether the differential growth occasioned by this difference in the incident light can account for phototropic curvature. It is clear from the data (Table 9) that the growth changes in the light-

TABLE 9.—COMPARISON OF THE LIGHT-GROWTH REACTION IN THE AVENA COLEOPTILE SUBJECTED TO GENERAL ILLUMINATION OF TWO INTENSITIES, AND GROWTH ON THE TWO SIDES OF A SIMILAR COLEOPTILE UNILATERALLY ILLUMINATED SO THAT THE LIGHTED AND SHADED SIDES ARE SUBJECTED TO THE SAME TWO INTENSITIES

Difference	After 1 hr.	After 2 hr.	After 2½ hr.
In growth reactions of 2.5 and 80 m.c.....	7.8	15.9	19.6
Between the convex and concave sides of a curvature at 80 m.c.....	22.1	67	83

growth reactions under the two different intensities are not sufficient to produce a curvature of the size that is actually obtained in the unilaterally illuminated coleoptile. With a somewhat different approach, Beyer came to the same conclusion. In Beyer's experiments, three series of plants were illuminated bilaterally for one hour. At the end of the hour, the illumination was continued unchanged in series A; in series B, both lamps were turned off; in series C, one lamp was turned off. Phototropic curvature resulted in the plants of the C series by decreasing and not by increasing the light intensity. According to the Blaauw theory, one would expect that the lighted side C_l of the curved plants should grow just as fast or certainly not any more slowly than that of a plant in series A and that the shaded side C_s should grow as fast as, or no faster than, a plant in series B. The results may be expressed in the following way: $A - C_l = 0.07$ and 0.08, and $C_s - B = 0.12$ and 0.11. Since a decrease in the rate of growth takes place on the lighted side, while an increase occurs on the darkened side, the data are not in accordance with Blaauw's theory. Bergann's (1930)

An important question is whether or not the phototropic stimulus can be transmitted in an acropetal direction as well as in a basipetal one. Rothert (1894) and van der Wolk (1911) found that it could not, but von Guttenberg (1913) demonstrated that, when coleoptile tips of plants whose basal portions had been previously illuminated unilaterally were lighted from the opposite side, the tip curvature which might have been expected was either absent or exhibited feebly. The author concluded that a stimulus is conducted from the unilaterally illuminated basal portion to the tip. This is not consistent with the investigations on growth-substance transport which have shown that its transmission takes place only in a basipetal direction. Arisz (1915) criticized the work of von Guttenberg on other grounds. Lange (1927) suggested that during unilateral illumination of the base, some of the light might fall upon the tip and in this way influence the transmission of stimulus. Reinhard and Bro (1933) have pointed out still other possibilities.

Quantity-of-stimulus Principle.—It has been held for a long time that in certain photochemical reactions the amount of applied energy is of importance in bringing about a constant effect. Bunsen and Roscoe (1862) proposed a quantitative rule for the effect of light upon a sensitive photographic plate, where the product of the exposure time $\times$ intensity of light $=$ a constant value.

Fröschel (1908, 1909) and Blaauw (1909) showed that a definite amount of light must be applied to a plant organ in order to produce a threshold phototropic response. The amount of light is the product of two factors: the intensity of light and the duration of illumination. That the quantity-of-stimulus principle holds over a wide range for phototropic curvature in the Avena coleoptile may be seen in Table 6, which is taken from the work of Blaauw. The fact that such a short illumination as 1/1,000 second can call forth a phototropic curvature is of importance for the comprehension of the induction process. Certainly the matter of photic stimulation is an excellent example of the general rule regarding the excitation of irritable protoplasm, *i.e.*, that a relatively small amount of applied energy is capable of setting into action a chain of processes which leads eventually to a comparatively large response. The significance of the Blaauw theory will be discussed in greater detail later on.

TABLE 6.—THE QUANTITY-OF-STIMULUS PRINCIPLE IN THE PHOTOTROPIC RESPONSE OF THE AVENA COLEOPTILE
(*Blaauw*, 1909)

Illumination, meter-candles	Duration of illumination	Amount of light, meter-candle seconds
0.00017	43 hours	26.3
0.000439	13 hours	20.6
0.000609	10 hours	21.9
0.000855	6 hours	18.6
0.001769	3 hours	19.1
0.002706	100 minutes	16.2
0.004773	60 minutes	17.2
0.01018	30 minutes	18.3
0.01640	20 minutes	19.7
0.0249	15 minutes	22.4
0.0498	8 minutes	23.9
0.0898	4 minutes	21.6
0.6156	40 seconds	24.8
1.0998	25 seconds	27.5
3.02813	8 seconds	24.2
5.456	4 seconds	21.8
8.453	2 seconds	16.9
18.94	1 second	18.9
45.05	2/5 second	18.0
308.7	2/25 second	24.7
511.4	1/25 second	20.5
1,255	1/55 second	22.8
1,902	1/100 second	19.0
7,905	1/400 second	19.8
13,094	1/800 second	16.4
26,520	1/1,000 second	26.5

Along with a knowledge of the amount of light necessary to produce a threshold curvature, it has been found equally profitable to study the course of phototropic curvatures with greater amounts of light. Different observers have found that as the amount of light is increased progressively above the threshold-stimulus value, various types of positive and negative bending may occur.

Positive and Negative Curvatures.—Pringsheim (1909) observed that when an Avena coleoptile is unilaterally illuminated with increasing amounts of light, no curvature is produced so long as the amount of light is below the threshold. Above the minimum

quantity of light necessary for excitation, positive curvatures are evoked; *i.e.*, the stimulated organ bends toward the light over a considerable range of stimulus values. These curvatures become more pronounced and disappear less rapidly with increasing amounts of light. If the amount of light is increased still more within a certain range, a stage of indifference appears, and after some time an opposing movement may take place so that weak negative reactions can be observed. With still larger amounts of light, positive curvatures are again brought about. Pringsheim presented graphically the influence of the amount of applied light upon the manner of reaction of the seedlings, and these conclusions were verified by Clark (1913). It is clear from the investigations of Pringsheim and Clark that three different modes of response can be distinguished in the Avena coleoptile: the first positive curvature, the first negative curvature, and the second positive curvature.

Arisz (1915) investigated carefully the manner of reaction of the Avena coleoptile to different amounts of light in an attempt to determine at what quantities of light the various curvatures appeared. The results of his experiments (Table 7) indicate that

TABLE 7.—SIZE OF CURVATURE IN THE AVENA COLEOPTILE IN RESPONSE TO DIFFERENT AMOUNTS OF UNILATERAL LIGHT

Amount of Light, m.c.s.	Size of Maximum Curvature*
7.6	0.7
12.4	1
18.1	1.6
26.4	2.3
45	3
65	3.3
75	4
100	5
140	4.7
237	5.4
560	4
1,500	3
2,800	1.2

* Curvature in millimeters deviation from the vertical position. Coleoptiles were about 25 mm. in length.

the magnitude of curvature increases with the amount of light applied until a maximum curvature is reached with about 100 meter-candle seconds. After this point, curvature decreases with increasing amounts of light. With about 6,000 to 40,000 meter-

candle seconds, the first positive curvature disappears entirely, and a pure negative curvature appears. With still greater amounts of light, the negative curvature also disappears, and a positive curvature appears immediately—the so-called second positive curvature. The negative curvatures appear only when the tip is illuminated and cannot be definitely demonstrated when the base is unilaterally illuminated.

These results have been confirmed by other investigators. Lundegardh (1922) found that the first purely positive reaction appeared with light values up to 10 meter-candle seconds, the maximum being at 10 meter-candle seconds. The range of the negative curvature lay between 800 and 500,000 meter-candle seconds, with a minimum at 4,000 to 10,000, the second maximum being at about 2,000,000 meter-candle seconds. The reaction decreased again with still greater amounts of light.

The experiments of Pringsheim, Clark, Arisz, and Lundegardh were all carried out with mixed white light. Du Buy and Nuernbergk (1929*b*) investigated the course of phototropic response using monochromatic blue light of wave length 4,360Å. Only the apical 2 to 3 mm. portion of the coleoptile was illuminated. The amount of light was measured in ergs per square centimeter per second. For the sake of comparison with the other investigations cited, 1 erg at wave length 4,360Å. may be considered as corresponding to about 10 meter-candle seconds (Table 8). Du Buy and Nuernbergk believe that they are able to distinguish

TABLE 8.—POSITIVE AND NEGATIVE CURVATURES IN THE AVENA COLEOPTILE IN RESPONSE TO DIFFERENT AMOUNTS OF RADIATION

Seconds	Ergs	Response	Curvature
1/50	12.2	Soon, very obvious	+
1/25	24.2	Soon, fairly obvious	+
1/5	122	Later, weak	−
1	610	Later	−
5	3,050	Later, weak	+
12	7,320	Soon, weak	+
25	15,250	Later	−
100	61,000	Soon	−
300	183,000	Soon, good	+
600	366,000	Later, weak	+
900	549,000	Later, good	+

five types of curvatures, *viz.*, three different positive curvatures, which are separated from each other by two different negative curvatures, or indifferent stages. A comparison of the data given in Table 8 with the results of Arisz shows that the second positive curvature of du Buy and Nuernbergk is new, appearing first at about 30,000 to 70,000 meter-candle seconds, *i.e.*, in the range that, according to Arisz and Lundegardh, gives negative curvatures. The first positive curvature of du Buy and Nuernbergk corresponds to the first positive curvature of Arisz, and the third positive curvature of du Buy and Nuernbergk corresponds to the second positive curvature of Arisz.

The Primary Positively Phototropic Curvature.—Some analyses of the growth processes which occur during the curvatures will now be reviewed. It can be shown experimentally that the first positively phototropic curvature is not connected with a change in the average rate of growth. For such experiments, Cholodny (1930) used a micropotometer. An excised Avena coleoptile from which the primary leaf had been removed was plugged with lanolin at the bottom and placed in the enlarged opening of a capillary tube bent at right angles so that the intake of water could be measured over a period of time. The coleoptile was placed in a saturated moist chamber to check transpiration. It was assumed that all the water taken up under these conditions was used for the volume increase of the coleoptile during growth, and the intake of water, therefore, was used as a measure of the growth rate. It was found that no appreciable change in the rate of growth could be shown in coleoptiles that were unilaterally illuminated with a light value of 500 to 2,000 meter-candle seconds and afterward darkened, although decided phototropic curvatures resulted in the course of 1½ to 2 hours. Du Buy and Nuernbergk obtained similar results at a later date. In these investigations, the course of growth of both the front and back sides of an Avena coleoptile was recorded during the first positive curvature produced by unilateral illumination of the tip with 3.55 ergs at a wave length of 4,360Å. for 2 seconds. From the curves it may be seen that the average rate of growth of the coleoptile is not changed during the curvature, so the first positive curvature involves an increase in the rate of growth of the back side and a corresponding decrease in the growth of the lighted side. The increase and decrease in the rate of growth of

the two halves just balance, so that no change in the growth rate of the whole organ takes place under conditions leading to phototropic curvature.

The course of the first positive curvature has been investigated by Lundegardh (1922), Went (1928*b*), du Buy and Nuernbergk (1929*b*), Dolk (1929*b*), and du Buy (1933). Dolk illuminated Avena coleoptiles unilaterally with 50 meter-candles for 10 seconds and recorded the course of the tropic curvature cinematographically. The radii of curvature of the individual growth zones were measured on enlarged photographs. The results of his investigations are given in Fig. 46. The ordinates give the size of curvature, *i.e.*, the reciprocal value of the radius of curvature, and the abscissae represent the time in minutes. The curvature begins almost simultaneously in the first three zones, that is, about 40 minutes after illumination, and proceeds downward. In the tenth zone, curvature becomes apparent only after 100 minutes. In the meantime, the curvature has increased continuously in the apical region, reaching its maximum in the first two zones after 170 to 180 minutes. That the average rate of growth is not changed during the second positive curvature has been shown by the investigations of Beyer (1927*c*), who employed three series of experimental plants: series *A*, illuminated bilaterally 1 hour and bilaterally 2 hours; series *B*, 1 hour bilaterally illuminated and 2 hours darkened; and series *C*, 1 hour illuminated bilaterally and 2 hours unilaterally (50 candles at a distance of 60 cm.). The growth of the lighted and darkened sides of series *C* during the phototropic curvature was then compared with that of both the other series. The linear growth in series *A* was 0.20 cm.; and in series *B*, 0.23 cm.; in *C* on the light side it was 0.11 cm., and on the dark side, 0.33 cm., which gives an average of 0.22 cm. The average rate of growth during phototropic curvature appears to be the same as it is in plants in the dark or in those bilaterally illuminated. Du Buy and Nuernbergk (1929*b*) obtained similar results when they followed the course of the second positive curvature under strong illumination. Although the second positive curvature corresponded to the course of the first positive response, a sudden increase in growth could be observed during illumination. The authors attributed this increase to cell-wall extension brought about by the strong illumination.

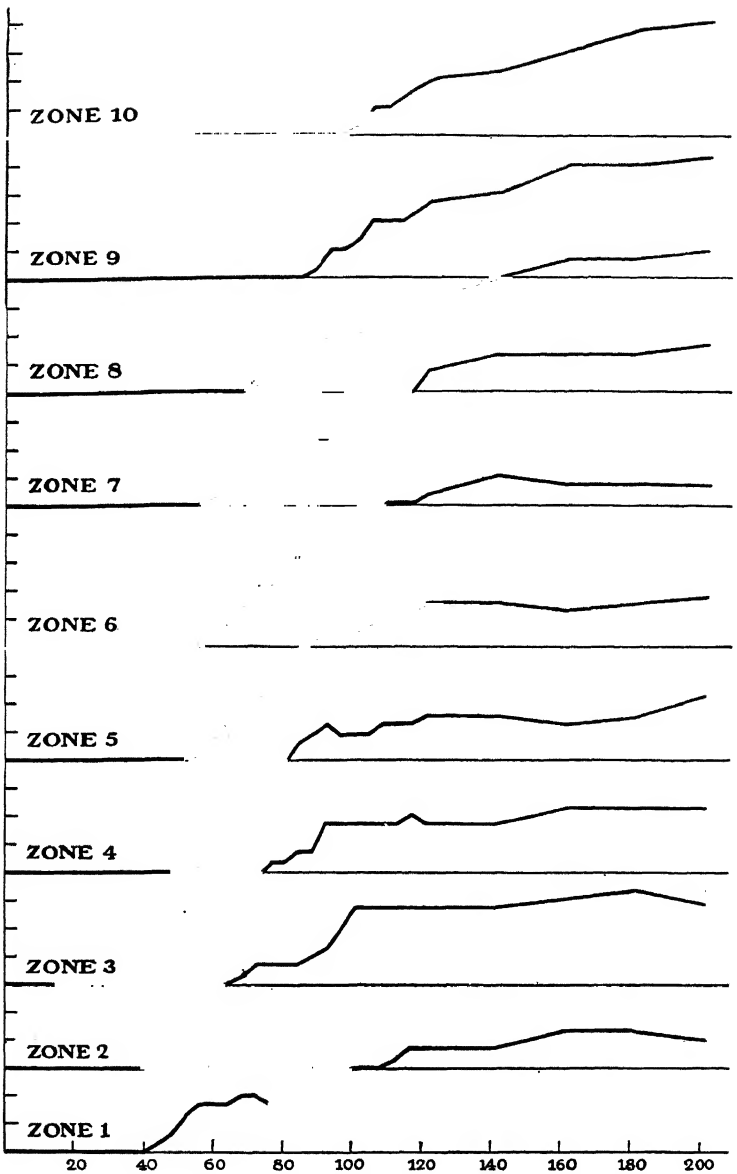


FIG. 46.—Course of curvature in different zones of the *Avena* coleoptile afte phototropic stimulation with 50 m.c. for 10 seconds. Ordinate: size of curvature abscissa: time in minutes. Zone 1 is at the tip, zone 10 at the base. (*Fro Dolk*, 1929*b*.)

In conclusion, it can be said concerning the course of phototropic curvatures that (1) there are three (or five) different types of reaction, two (or three) positive and one (or two) negative curvatures; (2) the curvature begins in the tip and proceeds in a basipetal direction; (3) the average rate of growth is not changed during the primary positively phototropic curvature. During the second phototropic curvature there is, however, according to du Buy and Nuernbergk, a generally lessened rate of growth. Apparently there is no agreement concerning the precise influence of light upon the second positive curvature.

The Blaauw Theory.—Among the different theories which have been proposed to explain the phenomena of phototropic curvature, perhaps the best known is that of Blaauw. The Blaauw theory has been discussed so often and so thoroughly in phototropic literature that it seems unnecessary to treat it here in great detail. This theory has been regarded occasionally as an explanation for all curvature phenomena, but actually it was applied for a time only to the phenomena of phototropism, and even here decisive proof was not obtained. The main point of the theory is that phototropic curvature in the separate regions of a plant organ comes about by an unequal rate of growth which is caused by an unequal distribution of light in the organ. Each part of the plant grows separately, according to the amount of light with which it is provided. Blaauw (1919) has described his theory in the following words: "The light-growth reaction is the primary phenomenon; phototropism is the secondary one which necessarily follows from it, when locally different light-growth reactions arise from locally different conditions of illumination." The ideas of Blaauw coincide, in the main, with the theory proposed earlier by de Candolle (1832). The application of the Blaauw theory to phototropism in the Avena coleoptile is difficult, because of the transmission of the stimulus which causes a phototropic curvature to take place in a region of the organ which is not directly illuminated.

Evidence Supporting the Blaauw Theory.—The test of the validity of the Blaauw theory of phototropic curvature in the Avena coleoptile begins with the determination of whether or not a phototropic curvature can be derived from the summation of the light-growth reactions of the illuminated and shaded sides under conditions of unilateral lighting. Sierp (1921) investigated the

effect of light upon the rate of growth in the coleoptile and concluded that the Blaauw theory is an adequate explanation of the observed light-growth response. Brauner (1922) also found a general agreement between the course of phototropic curvature and the light-growth reaction for small amounts of light, thus supporting the validity of the Blaauw theory (also see Brauner, 1927*a*). In these experiments, however, the distribution of light in the coleoptile was not taken into consideration; therefore, these attempts to derive the laws of phototropic curvature from the light-growth reaction of the front side alone appear to be illogical. Evidence that might favor the Blaauw theory is to be found in the work of Bergann (1930). He observed that the order of phototropic effectiveness declines from the blue to the orange regions of the spectrum in the same way as does the light-growth response, which consists of a depression of the growth rate. Van Dillewijn (1927*a*) made quantitative determinations of the course of the light-growth reaction on the front and back sides of the coleoptile and attempted to describe the course of the phototropic curvature. In van Dillewijn's computations, the light-growth reactions for different amounts of light were determined on the assumption that the illumination of the dark side was about one-thirtieth of that of the light side. Computations of growth curvatures for various methods of illumination yielded good qualitative agreement between the experimental results and what might have been expected on the basis of Blaauw's theory.

The weakness in van Dillewijn's reasoning is the fact that he did not actually observe the phototropic curvatures computed from the light-growth reactions but compared the latter with the curvatures obtained by Arisz. When an accurate quantitative comparison is made between the phototropic curvatures which are to be expected from light-growth reactions and those actually appearing, no agreement between light-growth reaction and curvature can be demonstrated.

Evidence Opposing the Blaauw Theory.—Evidence in opposition to the Blaauw theory has been obtained by Lundegardh (1922), von Guttenberg (1922), Pisek (1926, 1928), Beyer (1927*c*, 1928*b*), Went (1928*a*), Boysen Jensen (1928), du Buy (1933), and others. Pisek determined the difference in the growth reactions of coleoptiles subjected to intensities of 2.5 and 80 meter-candle seconds and at the same time measured the difference in length between

the convex and concave sides resulting from unilateral illumination with 80 meter-candle seconds, after different reaction times. In this experiment, the front and back sides of the unilaterally illuminated coleoptile are exposed to the same two light intensities as the two coleoptiles mentioned, owing to the light gradient through it. This should yield information of value as to whether the differential growth occasioned by this difference in the incident light can account for phototropic curvature. It is clear from the data (Table 9) that the growth changes in the light-

TABLE 9.—COMPARISON OF THE LIGHT-GROWTH REACTION IN THE AVENA COLEOPTILE SUBJECTED TO GENERAL ILLUMINATION OF TWO INTENSITIES, AND GROWTH ON THE TWO SIDES OF A SIMILAR COLEOPTILE UNILATERALLY ILLUMINATED SO THAT THE LIGHTED AND SHADED SIDES ARE SUBJECTED TO THE SAME TWO INTENSITIES

Difference	After 1 hr.	After 2 hr.	After 2½ hr.
In growth reactions of 2.5 and 80 m.c.....	7.8	15.9	19.6
Between the convex and concave sides of a curvature at 80 m.c.....	22.1	67	83

growth reactions under the two different intensities are not sufficient to produce a curvature of the size that is actually obtained in the unilaterally illuminated coleoptile. With a somewhat different approach, Beyer came to the same conclusion. In Beyer's experiments, three series of plants were illuminated bilaterally for one hour. At the end of the hour, the illumination was continued unchanged in series A; in series B, both lamps were turned off; in series C, one lamp was turned off. Phototropic curvature resulted in the plants of the C series by decreasing and not by increasing the light intensity. According to the Blaauw theory, one would expect that the lighted side C_l of the curved plants should grow just as fast or certainly not any more slowly than that of a plant in series A and that the shaded side C_s should grow as fast as, or no faster than, a plant in series B. The results may be expressed in the following way: $A - C_l = 0.07$ and 0.08, and $C_s - B = 0.12$ and 0.11. Since a decrease in the rate of growth takes place on the lighted side, while an increase occurs on the darkened side, the data are not in accordance with Blaauw's theory. Bergann's (1930)

criticism of the matter will be mentioned later. Cholodny (1931*d*, 1932*b*, 1933*a*) has pointed out additional discrepancies between the light-growth reaction and phototropism. He demonstrated that coleoptiles, immersed in water, can curve phototropically with very brief periods of illumination, without showing any kind of light-growth reaction.

In an entirely different way, Boysen Jensen (1928) showed the inadequacy of Blaauw's theory for phototropism. The coleoptile was split lengthwise, and a rectangular platinum plate was inserted in such a way as to divide the organ into two halves, so that each could be illuminated by itself. If Blaauw's theory were applicable, a phototropic curvature should have been produced by suitably decreasing both light sources. The front half was illuminated with 51, the back half with 0.9, 1.6, and 2.6 meter-candles, so that the light decrease from the front to the back was about 51:1, 32:1, and 20:1. Only minimal phototropic curvatures were obtained. In other experiments, a glass plate was inserted instead of a platinum one, and the tip was illuminated unilaterally at right angles to the plate. Here, again, only a minimal phototropic curvature resulted, although the distribution of light was the same as in intact plants. These experiments show plainly that the phototropic curvature does not come about through separate reactions of the single parts of the tip.

To test the validity of Blaauw's theory, Li (1934) conducted experiments with decapitated Avena coleoptiles which were exposed to different amounts of unilateral light. It was found that immediately following decapitation, short exposures of 10 minutes even at 28,800 meter-candle seconds were incapable of inducing bending, while in exposures of 30 minutes curvature was caused by 3,600 meter-candle seconds. When the exposure was extended to 3 hours, even an intensity of 100 meter-candle seconds could elicit a response. It is quite clear that Blaauw's theory does not apply to decapitated coleoptiles.

In many cases, a rather far-reaching parallel can be found between the light-growth reaction and the course of phototropic curvature. By a method of compensation whereby the coleoptile is bilaterally illuminated, the stimulus values of different regions of the spectrum were obtained (Bergann, 1930). It was found "that illumination from all regions of the spectrum (except red and infrared), when applied in corresponding intensi-

ties, produce equal light-growth reactions. Different wave lengths, when applied in suitable intensities, also produce equal curvature reactions (first positive, negative, and second positive)." In spite of agreement of this sort, it must be said that phototropism and light-growth reaction in Avena are two fundamentally different processes. The reader is referred to Bünning (1929) for further discussion of the matter.

Conclusions in Regard to the Blaauw Theory.—It is highly probable that the light-growth reaction which appears in unlocalized illumination also exists in unilateral illumination and therefore may have some significance in the production of phototropic curvature. In view of the available data on the subject, it is certain that Blaauw's theory is not sufficient to explain the observed changes in the rates of growth during the phototropic response of the Avena coleoptile. It can be shown that phototropism is connected with the transverse transport of a growth substance, while such a phenomenon is not concerned in light-growth reactions which appear with unlocalized illumination.

The Growth-substance Explanation.—The historical development of the growth-substance explanation of tropisms has been sketched in some detail in the first chapter. Sachs (1882*a*) early postulated the existence of formative substances which were supposed to control growth and development in plants. Definite evidence in favor of special growth substances was not forthcoming until some thirty or forty years later when, in connection with certain studies on phototropism, it was demonstrated that a growth-promoting substance is dispersed from the tip of the Avena coleoptile. (See Figs. 1 and 2 for a graphical story of the discovery of growth substances.)

The Relation of Growth Substance to Phototropism.—In a paper entitled "Das Problem des Phototropismus und sein Ende," Blaauw (1919) wrote:

> The problem of phototropism in itself has become empty. Further theoretical observations on this problem will only keep us still further from the investigation of the actual and therefore significant phenomena of growth. Surely there is no problem in phototropism itself, since it is a pure growth phenomenon. Growth, however, as a phenomenon of life, is a problem of great depth.

The growth-substance explanation has been proposed for both phototropic and geotropic phenomena. The application of this

explanation to phototropic curvature in the Avena coleoptile is presented with emphasis upon the positive curvatures arising from unilateral illumination of the tip. Since the phototropic curvature in the basal region results from a difference in the rate of growth upon the back and front sides, and since also the growth of the basal region is known to be regulated by the growth-substance supply from the tip, it can be concluded that the curvature must arise as a result of more growth substance flowing down the back side than the front side.

The Question of Wound Substances.—The growth-substance explanation of phototropic curvature of the Avena coleoptile rests upon the proof that such a curvature can be produced by a growth-promoting substance, migrating down the shaded (or back) side of the coleoptile. After Paál and Söding showed that a growth-promoting substance migrates from the tip into the basal region in dark-grown plants, the question arose as to whether this growth substance was identical with the growth substance acting during the phototropic reaction or whether special tropism hormones—"tropohormones" (Cholodny)—exist. Since growth substances in the coleoptile could be demonstrated only by their effects upon the rate of growth, it was not easy to determine whether the observed phenomena were due to one growth substance or many.

Stark and Drechsel (1922) held that special tropism hormones exist. They carried out experiments on the transmission of the phototropic stimulus when excised tips of one species or genus were placed on the bases of other species or genera. When these were stimulated with unilateral illumination, it was found that bases with foreign tips applied reacted much more slowly than with tips of their own kind. It was concluded that the phototropic compatibility decreases with increased distance of natural relationship. These experiments were interpreted to mean that the stimulating substances are, to a certain degree, specific. Later, this hypothesis was advocated by Beyer (1928*a*) also, who held that no quantitative relationship exists between the regeneration of growth substance in a decapitated coleoptile and the restoration of the phototropic sensitivity.

Similar conclusions have been reached by Li (1934), who found that the decapitated coleoptile is sensitive to light immediately following decapitation and that "physiological regeneration,"

leading to increased sensitivity, is concerned only with the production of growth substance and bears no relation to phototropic sensitivity.

The existence of special tropohormones has been disputed by other investigators, *viz.*, Cholodny (1927) and Went (1928*a*). Cholodny emphasized the fact that the rate of growth during phototropic curvature remains unchanged, which indicates that during unilateral illumination the growth-substance production is unchanged also, and that probably no new substances are formed. Under critical examination, the experiments of Stark, Drechsel, and Beyer in no way demonstrate the existence of special phototropohormones. Cholodny (1929*a*) has pointed out that the experiments of Beyer are consistent with the assumption that there is but one growth substance in the Avena coleoptile.

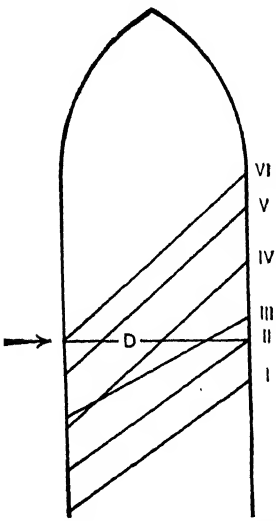


FIG. 47.—Diagram of the tip of an *Avena* coleoptile showing different ways in which unequal distribution of growth hormone might occur as a result of unilateral illumination. (*From Boysen Jensen.*)

It seems quite certain that no special photo- (or geo-) tropohormones are present in the Avena coleoptile, for experiments which are to be mentioned later show that unilateral light or gravity can produce a displacement of the growth substance and therefore an unequal distribution of it. These experiments show beyond question that photo- and geotropic curvatures are brought about by a growth substance which is also present in the tip of plants in the dark and not by special hormones. The question, however, as to whether or not other tropohormones, for example, chemotropohormones, are concerned in traumatic curvatures is not so easily settled.

Origin of the Unequal Distribution of Growth Substance.—The amount of growth substance given off by the tip in unilaterally illuminated plants as well as in plants grown in the dark is probably conditioned by the growth-substance concentration in the tip. If more growth substance is supplied by the tip to the

back than to the front side during the phototropic curvature, the growth-substance concentration upon the back side of the tip will be greater than that upon the front side. There are several other possible ways of explaining the origin of this difference in concentration. Some of these are shown in Fig. 47. The line *D* in the figure shows the growth-substance concentration in the tip of plants grown in the dark, while the different possibilities of its distribution which can lead to positive curvatures are shown by the lines I to VI.

Contrasting Theories of Boysen Jensen and Paál.—As a result of studies on the transmission of the stimulus in the Avena coleoptile, Boysen Jensen concluded that phototropic curvature is brought about by an increase in the rate of growth upon the back side of the coleoptile, caused by a downward migrating substance (line VI in Fig. 47). In opposition to this view, Paál (1918) presented the hypothesis that the phototropic curvature may be caused by a retardation of growth upon the front side. He held that the growth-promoting substances, which show an unlocalized migration from the tip of plants in the dark, are either partly destroyed by the light on the front side of the coleoptile tip or are impeded in their movement in such a manner as to produce a growth retardation upon the front side of the coleoptile. Paál's suggestion can be represented by the line I or II in Fig. 47.

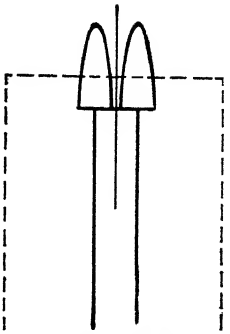


FIG. 48.—Diagram of a decapitated *Avena* coleoptile with two coleoptile tips applied symmetrically. A platinum plate is inserted vertically in the stump between the two tips. This permits illumination of a single tip from one side. (*From Boysen Jensen.*)

Paál's theory is not consistent with the investigations of Boysen Jensen, and Purdy (1921), who demonstrated that the transmission of the stimulus can be almost completely inhibited by a transverse incision upon the dark side. Furthermore, the theory is refuted by certain experiments of Boysen Jensen and Nielsen (1925). In these experiments with coleoptiles 2 to 3 cm. long, a 4 mm. tip and the upper portion of the primary leaf were removed. The empty part of each coleoptile was then split, and a thin, rectangular piece of platinum was inserted in the incision. Two coleoptile tips were then placed symmetrically

upon the coleoptile stump (Fig. 48), and one of these was unilaterally illuminated in the usual fashion. Curvature toward the light was produced as shown in Fig. 49. This experiment indicates that the flow of growth substance upon the darkened side is increased above the normal, and from this it follows that the growth-substance concentration upon the back side of the tip is increased by unilateral illumination.

Purdy's Theory.—What is known regarding the growth-substance concentration upon the front side of a unilaterally lighted tip? According to one hypothesis (indicated by line VI

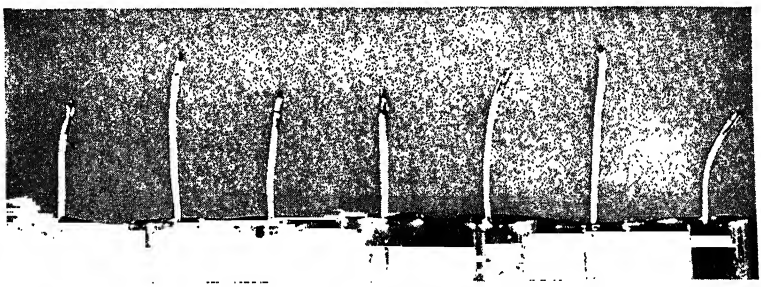


FIG. 49.—*Avena* coleoptiles as diagrammed in Fig. 48. Light came from the right-hand side, so only the tips on the right were illuminated; positive curvatures resulted. (*From Boysen Jensen and Nielsen*, 1925.)

in Fig. 47), the phototropic curvature may arise solely through the increase in the growth-substance concentration upon the back side, while it remains unchanged upon the front side. The experiments of Purdy, however, indicate that even when the transmission of the stimulus upon the back side is blocked, a slight phototropic curvature occurs. To explain this, one must assume that the rate of growth upon the lighted side is slightly retarded. According to this hypothesis (represented graphically by line V in Fig. 47), the growth-substance concentration upon the front side is slightly decreased.

The Went Theory.—Went (1928*a*) determined the amount of growth substance given off on the illuminated and shaded sides of unilaterally illuminated coleoptile tips by placing the tip upon two agar blocks, so that the lighted side stood upon one block and the darkened side upon the other. It was found that less growth substance was given off by the lighted side (about 46 per cent as much as from the darkened side). According to Went, the

distribution of growth substance in the illuminated tip should be represented by line III in Fig. 47.

Theories of Beyer, Cholodny, du Buy, and Nuernbergk.—The total concentration of growth substance present in the coleoptile during phototropic curvature, in relation to the normal concentration, is slightly increased according to the hypothesis of Boysen Jensen (as represented in line V). On the other hand, according to the hypothesis of Went (line III), the amount of growth substance is slightly decreased. The investigations of Beyer, Cholodny, and du Buy and Nuernbergk have shown that the average rate of general growth during phototropic curvature is not demonstrably changed, and one must conclude, therefore, that none of the foregoing hypotheses is entirely correct but rather that the growth-substance distribution in the tip during phototropic curvature is proportionately decreased on the illuminated side and increased on the shaded side, as represented by line IV in Fig. 47.

It has been shown that different types of phototropic curvature may occur in response to different amounts of light. The question arises as to whether or not the distribution of growth substance in the tip can be correlated with the two different positive curvatures. Since, according to du Buy and Nuernbergk, the rate of growth is unchanged in both curvatures, it may be concluded that the distribution of growth substance is substantially the same in both cases. What the growth-substance distribution may be during the negative curvatures, remains to be investigated.

The Displacement of Growth Substance.—The origin of the unequal distribution of growth substance during phototropic induction remains an important point for further discussion. Various suggestions have been offered to explain the growth-substance distribution as presented diagrammatically in line IV in Fig. 47. One might conclude that the rate of formation of growth substance in the tip is governed by the application of light, so that at certain light intensities the amount present is increased, while at other intensities it is decreased. However, such a notion would seem to refer us back to Blaauw's theory which has been proved inadequate as an explanation for phototropic curvature in the Avena coleoptile.

The assumption of an unequal distribution of growth substance brought about by an exchange of material between the light and

dark side of the coleoptile tip is supported by certain of Boysen Jensen's investigations (1928). When the tip of an Avena coleoptile was split and a thin glass plate was inserted into this incision parallel to the direction of light, normal phototropic curvature resulted in response to unilateral illumination. On the other hand, the curvature was very weak when the plate was oriented perpendicularly to the direction of light. If both halves of the tip, without the insertion of a glass plate, were held together closely either with a platinum spiral or with a thin glass tube, a phototropic curvature took place even when the incision was oriented perpendicularly to the direction of the light beam. The result of this experiment can be explained only on the assumption that an exchange of material between the two halves of the tip takes place during the photic induction.

The question arises, What kind of substance is transported during the induction? At least two possibilities present themselves: It could be assumed that the growth substance itself was displaced in a transverse direction during the action of unilateral light. Or, on the theory that a constant formation of growth substance takes place in the coleoptile tip, it could be assumed that the growth substance itself was not transported, but rather another substance which in turn influences growth-substance production. The displacement of such a substance should then cause a lessened formation of growth substance upon the front side and an increased formation of growth substance upon the back side. Since both of the above were possibilities, the author did not venture to draw definite conclusions from his investigations, though the first of these two assumptions was without doubt the simpler.

At practically the same time as the publication of Boysen Jensen's (1928) experiments, Cholodny (1927) and Went (1928*a*) expressed the idea that the unequal distribution of growth substance in the coleoptile comes about by a growth-substance displacement. Cholodny proceeded with his investigations on the assumption that tropic stimuli do not influence the production of growth substances. Went supported his ideas with experiments which seemed to show that after phototropic induction, the amount of growth substance given off by the lighted side of the tip was lessened, while that of the darkened side was increased. The results of his experiments are given in Table 10

in a very reduced form. If the amount of growth substance given off by the tip, in darkness, is adjusted to equal 100 per cent, then, on this scale, the growth substance given off by the shaded side of a unilaterally illuminated tip is increased from the expected 50 to 57 per cent. By another method of calculating the results given

TABLE 10.—RELATIVE AMOUNTS OF GROWTH SUBSTANCE RECOVERED FROM AVENA-COLEOPTILE TIPS PLACED UPON AGAR BLOCKS AND KEPT EITHER IN DARKNESS OR EXPOSED TO UNILATERAL LIGHT
See Fig. 2 (Went)

Experiment number	In darkness	Illuminated with 1,000 meter-candle seconds			
		Time on agar, minutes	Lighted side	Shaded side	Total
360–363	100	70	38	57	95
364–367	100	60	26	51	77
368–374	100	90	6	62	68
379–381	100	80	32	60	92
383–388	100	120	33	57	90
Average.....	100	84	27	57	84

in Table 10, Went (1935) adjusted to 100 per cent the total amount given off into agar by the illuminated tip; then 65 per cent of this is dispersed into the block beneath the shaded side, and 35 per cent into that under the illuminated side (Fig. 2) (Went). There is some doubt whether one can conclude from these experiments that the amount of growth substance given off on the shaded side is increased by unilateral illumination. This method does not give entirely satisfactory evidence for the displacement of growth substance in the tip.

It does not seem possible to present proof for the displacement of growth substance in the coleoptile tip, since new substance is continually being dispersed from there. In the basal portion of a decapitated coleoptile, however, none is present for some time after decapitation, and it should be possible here to decide whether a displacement of growth substance can take place. Dolk (1929*b*) showed that in horizontal coleoptile cylinders of Avena which were supplied with growth substance at one end,

more growth substance was conducted through the lower than through the upper half. Although it is possible that unilateral changes in permeability may be produced by the action of gravity, whereby differences in the conduction of the substance might arise, still these experiments show decidedly that the growth substance can actually be displaced transversely by the action of gravity.

The effect of unilateral light upon the distribution of growth substance in the coleoptile stump of Avena has been investigated by Boysen Jensen (1933*a*). When an Avena coleoptile was decapitated, and the cut surface covered with a block of growth-substance agar, the growth substance proved to be unlocalized in its downward flow. When the upper part of such a plant was unilaterally illuminated, and the lower portion was darkened, a positive phototropic curvature arose not only in the upper, lighted portion but also in the lower, darkened portion. It would appear, therefore, that in the shaded part of the coleoptile, more growth substance flows downward along the back side than upon the front side. The difference in the conduction of growth substance upon the front and the back side of the lighted portion of the coleoptile could be explained by the destruction of the growth substance upon the front side, by the lowering of the permeability upon the front side, or by the displacement of growth substance from the front to the back under the influence of light. Displacement of growth substance was directly proved by a comparison of the phototropic curvature in coleoptile stumps with and without growth substance present. Some decapitated coleoptiles were covered with growth-substance agar and some with plain agar blocks for a sufficient period of time to permit the intake of substances present in the agar. Then the blocks were removed, and the coleoptile stumps were illuminated with continuous light. It was found that in coleoptile stumps receiving growth substance, the phototropic curvature appeared about 2 hours earlier than in those without growth substance. This result can be explained by the assumption that the growth substance present in the coleoptile is displaced in a transverse direction by the unilateral effect of light. In some instances, the growth-substance content of the coleoptile stump can be so great that a phototropic curvature does not appear, the reason being that an ample supply of the growth substance remains upon the front

side (in spite of its displacement) to produce a maximum rate of growth under the prevailing conditions. Although a displacement of growth substance in the coleoptile tip has not been proved directly, it may be supposed, on the basis of these experiments with subapical portions, that growth substance is displaced by the action of unilateral light in the tip.

Growth-substance Transfer and Electrical Potential.—Proof of the accumulation of growth substance upon the back side of the unilaterally illuminated tip has supplied a link in the chain of phototropic response, which may be considered as the concluding link in the process of induction. There remain for consideration the preceding factors in induction, *i.e.*, how the effect of unilateral light can produce a displacement of growth substance; and the subsequent factors, *i.e.*, how the unequal distribution of growth substance can produce the positive phototropic curvature in the Avena coleoptile.

A consideration of the first steps in the process of induction leads back to the problem of growth-substance transport in the plant. The difficulties of constructing a plausible theory for the longitudinal transport of growth substance have been discussed in Chap. V. It is equally difficult to explain the transverse transport of growth substance in a satisfactory way. In a discussion of the theory of transverse transport, it should be pointed out that illumination for a mere fraction of a second can produce a curvature. Effective displacement of growth substance naturally cannot take place so rapidly. It must be concluded, therefore, that illumination creates a condition in the coleoptile tip which is the primary cause of the displacement of growth substance.

Since growth substance is an acid, one might suppose that the unilateral accumulation of it could be brought about by differences in electrical potential induced by illumination of the tip. On this supposition, the back side would have to be electropositive with respect to the front side. According to the investigations of Waller (1929) and Bose (1928), a potential difference actually exists in unilaterally illuminated stems in such a way that the shaded side is positive. Whether the observed differences in potential are sufficient to explain the transverse displacement of growth substance is discussed more fully under geotropic curvatures.

Recently, Koch (1934) has demonstrated the transport of growth substance across the Avena coleoptile toward the positive side in an artificially produced electric field. Similarly, Kögl (1933, Mitt. VI) and Ramshorn (1934) have shown the electrical transport of growth substance in *Avena sativa* and other species of plants. There are various obstacles in the way of an electrical explanation. Not all organs that contain growth substances react phototropically. A theory adequate to explain the transverse transport of growth substance must take into consideration the negative as well as the positive responses.

Conclusion in Regard to the Growth-substance Explanation.— If an unequal distribution of growth substance has taken place in the tip itself, a curvature must of necessity follow in the lower portions of the organ, since growth-substance transport in the basal region takes place almost exclusively in a longitudinal direction. According to du Buy (1933), the following three factors must be taken into consideration for the evolution of a phototropic curvature: (1) the production of growth substance, (2) the transport of growth substance, and (3) the effect of growth substance. Actually, the methods that one has for the measurement of these individual components are still far from perfect. Even in the simplest case of unilateral illumination of the tip, the quantitative relationship between the curvature and the distribution of growth substance (not to mention the amount of light applied) does not hold. When the entire coleoptile is illuminated, the difficulties become still greater. The phototropic curvature is determined not only by the amount of growth substance given off from the tip but also by its displacement throughout the basal region. Other factors must be considered also, such as the effect of light upon the activity of the growth substance and modifications in the inherent capacity for response.

The importance of light absorption for phototropism may be emphasized by brief reference to the peculiar action of certain dyes introduced experimentally into the living tissues of plants. Blum and Scott (1933) have demonstrated the photosensitizing effect of dilute erythrosin upon wheat roots grown in a nutrient solution. In the presence of 1:500,000 erythrosin, the unilaterally illuminated roots exhibited relatively greater growth rates on the shaded side, so that bending occurred toward the

light. In the absence of dye, these roots were normally not phototropic. This response was attributed to the light absorption of the dye in a manner similar to that exhibited by the naturally occurring porphyrins in plants that are generally photodynamic.

On the other hand, the presence of certain dyes in plants has in some instances been found to destroy the phototropic response without having any great effect upon the average rate of growth (Boas, 1933; Schweighart, 1935). Boas found that seedlings of *Lolium perenne* treated with dilute eosin exhibited no phototropic curvature in response to unilateral illumination. The eosin seemed to affect both sensitivity to the stimulus and the distribution of the growth substance, though, obviously enough, the mechanism is still imperfectly understood.

The growth substance which is active in phototropic curvatures is identical with the growth substance of normal growth. By unilateral illumination of the coleoptile tip, the rate of growth-substance dispersal is scarcely changed, but a displacement of it in a transverse direction takes place with the result that its concentration is greater upon the shaded than upon the illuminated side. The flow of growth substance into the basal region on the front side of the coleoptile is decreased, and that upon the back side is increased; therefore, the rate of subsequent growth on these two sides is roughly proportional to the amounts of controlling growth substance present. The average rate of growth over-all is either not changed or only slightly altered.

Blaauw's theory assumes that the individual parts of the coleoptile grow at rates inversely proportional to the differential amounts of light and that the phototropic curvature results from separate reactions of the individual regions. According to the growth-substance explanation, or theory, the coleoptile tip reacts as a whole, and a difference between the lighted and shaded sides of the organ is created by a displacement of growth substance in the unilaterally illuminated tip. Herein lies the fundamental difference between the two theories. The growth-substance theory properly interpreted is capable of explaining all the observed facts of phototropic response in the Avena coleoptile.

Other Theories on Phototropism.—The theory of Fitting (1907), mentioned in the introduction, holds that a polarization

of the single cells of the organ of perception arises from unilateral illumination and that this polarization is conducted along paths of living tissues to the region of reception. This theory might be questioned because, during phototropic induction, not only are the single cells polarized but also the tip as a whole. The recent findings of Rosene (1935) lend support to the previously expressed idea that the electrical potentials observed in plant organs result by the algebraic summation of the electromotive forces of polarized cells.

Brauner (1922, 1924) assumed that the phototropic curvature comes about by retardation of growth upon the illuminated side of the organ. He concluded that unilateral illumination increased the permeability of the lighted side and in this way promoted migration of the substances from their source at the tip down the front side, producing a retardation of growth on this side. The theory fails because the substances dispersed from the tip have a promoting and not an inhibiting effect upon the growth of the coleoptile. However, the fact remains that modifications in tissue permeability do occur as a result of illumination (Brauner 1924, 1935), and it is conceivable that the translocation of growth substance may be influenced thereby.

According to Priestley and Tetley (Priestley 1926*a*, *b*, *c*, *d*; Tetley and Priestley, 1927), the growth changes brought about by decapitation are not caused by growth substances but by changes in water supply. It was argued that cell elongation is conditioned by water intake. When the coleoptile is decapitated, water is exuded, and growth is decreased until the original conditions are again obtained by healing of the wound. Phototropic curvature was held to arise "by the increased resistance to stretching induced by the action of light upon the walls and by the increased entry of water and rapidity of the movement of sap generally, which is the result of the increased tissue permeability that follows exposure to light."

Tetley and Priestley close their discussion with the following words: "There seems no necessity to assume that any hypothetical substances are diffusing from the apex, and until such hormones are experimentally demonstrated they may quite well be dispensed with in theories of tropic response." This requirement is now fulfilled, and Priestley's explanation is no longer tenable.

Gradmann (1930) proposed a complicated theory of growth and of phototropic curvature which, in the main, is in accord with the growth-substance explanation. However, Gradmann's theory assumes the presence of two different growth substances. One of these substances (A) is formed only in the tip and flows in an unlocalized fashion into the basal region. It is identical with the growth substance described above. The other (B) arises along the entire length of the coleoptile and on up into the tip. Each of these substances alone is considered as being ineffective, but together they form a substance (AB) which increases growth. Photo- and geotropic stimuli cause more growth substance B to be formed on the shaded, or under, side; hence more of the compound AB is produced, thus increasing the rate of growth upon this side. With the increase of substance B in a particular region, a greater use of A follows. A flows to this region of deficit, and the diversion of the stream of growth substance A strengthens the primary effect considerably. With this hypothesis, Gradmann tried to combine Blaauw's theory with the growth-substance explanation, to account for the phototropic response. In accord with Blaauw, the unequal origin of B is the primary reaction, and it should be sufficient in itself to bring about a curvature.

The main objection to Gradmann's theory is that growth and growth changes in the Avena coleoptile can be much more easily explained by the growth-substance explanation alone. The Gradmann postulate is directly refuted by Boysen Jensen's (1933*a*) experiment in which phototropic curvature was hindered by the application of very great amounts of growth substance although the coleoptile grew vigorously. In spite of the displacement of growth substance, a sufficiently large amount of it remains on the front side to produce a maximum rate of growth permitted by the prevailing conditions. This experiment could not be explained, by Gradmann's theory, because if the curvature came about by unequal distribution of the substance B, then an excess of substance A should not hinder the phototropic curvature.

DICOTYLEDONOUS STEMS

The great majority of investigations on the question of phototropism have dealt with the Avena seedling, and, without doubt, many of the conclusions reached in these studies concerning

phototropic stimulus and response apply equally well to phototropism in other plants. Many valuable contributions to our knowledge of phototropic curvature, particularly in recent years, have developed out of studies on the response of dicotyledonous shoots to light.

Distribution of Phototropic Sensitivity.—Darwin found long ago that only the tip of the grass coleoptile was sensitive to light and that this apical region determined the phototropic curvature of the lower portion. Similar results were obtained also with the stems of *Brassica oleracea*. However, Darwin's conclusions were not entirely convincing because of the considerable variability in his experiments. Later on, Rothert (1894) came to different conclusions regarding the phototropism of stems of *Brassica napus*, Agrostemma, Vicia, and other dicotyledonous plants. The sensitivity to light was found to be irregularly distributed in the stem, being particularly strong in a relatively short region near the tip and weaker in the basal region. This differential distribution of light sensitivity was found to decrease with age in Vicia. In other seedling stems, such as those of Tropaeolum, Solanum, and Coriandrum, the sensitivity appeared to be regularly distributed over the stem, while the condition in Daucus and Linum formed a transition between these two groups.

Transmission of Phototropic Stimulus.—Rothert demonstrated the transmission of a phototropic stimulus in various seedling stems of dicotyledonous plants. When the upper region of the stem of *Brassica napus* was unilaterally lighted, and the lower region was darkened by dry earth, paper aprons, or paper tubes, phototropic curvatures appeared in the darkened region of the stem. Similar results were obtained with Agrostemma, *Tropaeolum minus*, and some other species. It was relatively difficult to demonstrate any transmission of stimulus in Vicia. A study of Rothert's photographs for the conduction of the phototropic stimulus shows that the curvatures which are obtained in the darkened basal region in dicotyledonous seedlings are smaller than those obtained in Avena similarly treated. It appears, therefore, that the region over which the stimulus is transmitted is comparatively small in these plants. A decided phototropic stimulus transmission, extending over several centimeters and at times of marked strength, has been demonstrated in the stems of *Linum usitatissimum*, *Brodiaea congesta*, and *Galium purpureum*.

The results of these experiments make it probable that growth substances, at least in certain cases, are also concerned in the phototropic response of stems.

Growth Substance and Phototropism in Seedling Axes.—Van Overbeek (1933), as mentioned before, studied the significance of growth substance for normal growth and the photogrowth reaction in *Raphanus sativus*. He demonstrated the formation of growth substance in the cotyledons and its subsequent movement into the hypocotyl. When the upper end of a hypocotyl cylinder was completely covered with growth substance and unilaterally illuminated, more growth substance was extracted from the basal regions on the dark side than on the lighted side (Fig. 2). This shows that it was displaced by the action of unilateral light, just as it is in the Avena coleoptile. It seems reasonable to conclude that the growth substance distributed from the cotyledons is displaced toward the back of the hypocotyl by unilateral light.

In addition, van Overbeek showed that the light-growth reaction was very strong in dark-adapted plants. With general illumination he found a growth retardation which amounted to over 50 per cent. This phenomenon could be explained neither by changes in growth-substance transport nor by the destruction of growth substance through the action of light, and van Overbeek assumed that the tonus of the seedling stem with reference to growth substance must be changed by the application of light. The importance of this phenomenon for phototropism in seedlings is clear. Under conditions of unilateral illumination, the light intensity on the lighted side was 5.2 times greater than that on the shaded side of the Raphanus hypocotyl. Since the rate of growth is retarded so greatly by the application of light, then unilateral illumination must be sufficient to produce a phototropic curvature in accord with Blaauw's theory. Boysen Jensen (1936) extracted with chloroform the growth substance from the front and back sides of unilaterally illuminated seedling axes of Phaseolus. During phototropic curvature the growth substance was more concentrated on the side away from the light.

Van Overbeek (1932) was able to show that the quantity of growth substance transported through a portion of Raphanus hypocotyl was about the same in the light as in the dark. The presence of the tip was not essential for bringing about a lateral

displacement of growth substance with illumination on one side, for it was found that the substance could be drawn toward the shaded side of a decapitated hypocotyl cylinder.

This investigator came to the conclusion that the phototropic curvature of the Raphanus hypocotyl is brought about by the combined action of two different effects: (1) by the differential retardation of growth, which is inhibited more upon the lighted side, and (2) by the displacement of growth substance to the back, which must produce an increase in growth upon the dark side and a retardation of growth upon the lighted side. He concluded, further, that the Blaauw theory and the growth-substance theory are not to be considered as antitheses but that the fundamental ideas of both are complementary.

The question remains how these two effects share quantitatively in producing the curvature. The unequal distribution of growth substance and of light must be considered together with the fact that the retarding effect of light is influenced by the growth-substance concentration. Although the computations become somewhat involved, it may be concluded that the curvature comes about mainly through the fact that growth on the lighted side is checked markedly, while growth of the darkened side is slightly increased in comparison with the normal.

THE PHYCOMYCES SPORANGIOPHORE

The growth-substance explanation has not yet been applied to positive phototropism in the sporangiophores of Phycomyces. Another explanation, however, has been attempted on the basis of the unequal distribution of light within the cylindrical stalk bearing the sporangium. Blaauw (1914, 1919) studied the light-growth reaction of Phycomyces and found that illumination increased the rate of growth. Wiechulla (1932) found that colored lights of equal phototropic effectiveness produce about the same size and type of accelerative growth response. Blaauw explained the positive (not negative) response which follows unilateral illumination, by pointing out that the parallel rays of light are concentrated on the back side owing to the lenticular effect of the sporangiophore. In agreement with this theory is the well-known experiment of Buder (1918), who immersed the fungus in paraffin oil to overcome the lens effect and cause the front side to be more strongly illuminated than the back side. A

negative instead of a positive curvature resulted under these conditions. According to Oehlkers (1926), the curvature is not brought about by any concentration of the light upon the back side but rather by the fact that the light rays penetrate farther in the back than in the front.

Castle (1930, 1933*a*, *b*) has investigated the whole question thoroughly. His computation of the path of light rays in the *sporangiophore* led to the conclusion that the light in the back half, owing to refraction, is 1.26 times as great as it is in the front half of the organ. When the absorption coefficient is not greater than 6, more light is absorbed in the back half of the cell than in the front. From this, Castle concluded that the difference in the amount of light absorbed causes the back side to grow more strongly than the front side; hence bending occurs toward the light.

Up to the present time, nothing is known concerning the significance of growth substance for the phototropic curvature of sporangiophores. It can be demonstrated easily that Phycomyces forms growth substance in culture, and it is possible, therefore, that this substance may be concerned in the phototropic curvature exhibited by the fungus. Heyn (1935) has extracted a growth substance from the sporangiophores of *Phycomyces nitens* which, on the basis of the coefficient of diffusion, appears to be 3-indole acetic acid.

SUMMARY

The observations of de Candolle (1832) led to an appreciation of the fact that differential growth is involved in the phototropic and geotropic responses of plants. These tropisms may be regarded as specialized cases of normal growth, where unequal stimulation on the two sides of an organ leads to greater enlargement on one side. The bending of plant organs toward light is the result of more rapid growth on the shaded side than on the side toward the light.

Since plants elongate less rapidly in the light than in darkness, Blaauw and others concluded that light must have a retarding effect upon growth. Subsequent detailed investigations of phototropic curvature in the Avena coleoptile and organs of other plants showed that (1) bending can be induced in the darkened part of an organ only when some other portion is illuminated; (2) the depressing action of light upon growth is not sufficient to

account for the curvatures induced by the same amount of unilateral light; (3) when the apical portion of an erect coleoptile is separated into two halves by the median lengthwise insertion of a thin glass plate oriented at right angles to the beam of incident light, the characteristic phototropic curvature does not occur. Such evidence demonstrated clearly that the Blaauw theory is inadequate to account for phototropism.

Experiments performed by many different investigators indicated that some chemical substance must be responsible for tropic growth. Such a conclusion was substantiated by experiments which showed that unequal growth on the two sides of a light-stimulated organ is due to unequal concentration of hormone on the two sides. Since the average rate of growth in length did not change during phototropic curvature, it followed that the active concentration probably was not affected by light.

Further investigations proved that the concentration of the growth hormone is decreased on the illuminated side and increased on the shaded side of the bending organ. The growth-hormone explanation of phototropism, based on studies of the Avena coleoptile, may be summarized as follows: The hormone is distributed from the distal end of the organ and flows downward into the elongating regions below. Unilateral illumination scarcely affects its formation but brings about displacement toward the shaded side during the course of its downward movement. The subsequent rate of growth on each side is proportional, within limits, to the concentration of the hormone present.

The displacement of growth substance in a direction away from light has been demonstrated also in the seedling stems of certain dicotyledonous plants. It has been shown, in addition, that its role in promoting cell elongation is hindered by light.

The mechanism by which light exercises a controlling influence upon growth-hormone distribution and activity is not well understood, but one is led to the conclusion that phototropism results from (1) a direct retarding effect of light upon growth, possibly brought about by its influence upon the molecular structure of cell walls, and (2) the differential accumulation of growth hormone within the organ. The directional movement of growth hormone leading to this differential accumulation is brought about by light through its influence on protoplasmic streaming, permeability, and electrical potential.

CHAPTER IX

THE SIGNIFICANCE OF GROWTH SUBSTANCES FOR GEOTROPISM

The bending of the organs of a plant toward or away from the earth is a well-known phenomenon. This response to gravity eventually brings about a position of equilibrium with respect to the earth. Perception of the gravitational stimulus and the course of the processes which lead to the geocurvature have long been the subject of scientific inquiries. Much of the older literature pertinent to the question of geotropism has been discussed in reviews by Schober (1899), Christiansen (1917), Zimmermann (1927), and Rawitscher (1932). Since much that is known about growth substances was learned in the study of tropisms, a few of the more outstanding original contributions will be discussed for purposes of orientation in this field.

THE EARLY INVESTIGATIONS

Knight (1806, 1803–1812) demonstrated by experiments in centrifuging that the force of gravity caused an upward bending of stems placed in a horizontal position and a downward curvature in horizontally placed main roots. He attempted to explain how the same stimulus could produce these opposite responses in the shoot and the root on the basis of a purely mechanical effect of gravity. Growth in the root takes place by the continual laying down of new tissues at the vegetative tip. In seedlings, the food for growth comes in solution from the cotyledons. It was assumed that gravity affected this fluid and the tender, flexible fibers and bundles in such a way as to cause bending of the root tip in a downward direction. The curvature was supposed to be a passive, downward movement of the tip, due to its weight. Such a hypothesis cannot be used as an explanation for the upward curvature of the stem, for Knight pointed out that it grows by the stretching of the region back of the tip, the amount of extension being in proportion to the food supply. It was held that an

accumulation of food takes place on the lower side of the horizontally placed stem owing to the effect of gravity and that this causes more growth upon the lower than upon the upper side. A negatively geotropic curvature results.

The theory did not stand under experimental tests, for Johnson in 1828 showed that during geotropic curvature the root can overcome a resistance which is at least one hundred times the weight of the root tip. Furthermore, Pinot found in 1829 that a root can bend into mercury, in spite of the fact that the specific gravity of the root tip is much less than that of the mercury. It may be seen from these investigations that work is performed in the positive geotropic curvature of a root and that this curvature is active rather than passive.

In spite of these investigations, Knight's theory was taken up anew from the anatomical standpoint by Hofmeister more than fifty years later. No anatomical differences between the root and the stem could be found as a basis for explaining these different reactions. Hofmeister concluded that the cause must be some fundamental difference or differences in regions where the curvatures actually occur. In the root, the zone in which the downward bending takes place is made up of similar cells which constitute "a region capable of curvature following the effect of gravity like a drop of viscous liquid." On the other hand, the portion of the stem which curves geotropically consists of differentiated elements which are stretched taut next to each other. Hofmeister (1867) concluded from a few experiments that the upward geotropic curvature of the stem probably results from the fact that "in the lower longitudinal half of the organ, the extensibility of the cell membranes is increased." The explanation of increased extensibility is that when the stem is in a horizontal position, more water can penetrate the membranes of the cells on the underside than those on the upper side.

Frank (1868 *ff.*; see Rawitscher, 1932) and other investigators opposed this hypothesis of Knight and Hofmeister, because they were convinced that the downward curvature of the root is an active process. The experiments and conclusions of Johnson and Pinot formed the basis for a long controversy which was finally decided in favor of Frank. The principal point of Frank's theory is that positive as well as negative geotropic curvatures are vital processes which result from changes in growth.

This interpretation of geotropic curvatures, established by Frank, was then rounded out by Sachs (1873–1882), as the result of numerous investigations which form the foundation of our knowledge of geotropic processes. Sachs evolved the general concept that any valid explanation of geotropism must explain both positive and negative curvatures. His idea may be stated as follows: A theory of geotropism is satisfactory only when it explains both positive and negative curvatures at the same time; when it shows why the same external cause produces the opposite effect in structurally similar cells and organs, *i.e.*, the promotion of growth upon the underside in the stem and inhibition of growth upon the underside in the root. The effect of gravity upon root and stem can be explained only by the assumption "that the inner organization (even though submicroscopic) of the different regions determines the different manner of reaction to the same external stimuli."

Sachs formulated a theory to account for opposite reactions of the root and stem in the plant. The geotropic processes he viewed as stimulation phenomena whose course is determined partly by external force and partly by the organization of the plant organ concerned. If the course of a stimulus phenomenon is determined by the internal organization of the plant, then the final response must be the result of the combined action of various single reactions in a chain. The problem is to analyze the chain of reactions which begin with the direct effect of gravity upon the plant cells and end with a positively or negatively geotropic response. The contribution of the theory of growth substance to the analysis of geotropism will be taken up for different plants separately, in the same manner as has been done for phototropic curvatures.

THE AVENA COLEOPTILE

The Avena coleoptile is negatively geotropic, because when displaced from the vertical position it grows away from the center of the earth. This geotropic curvature is the result of unequal rates of growth on the upper and lower sides of the displaced organ.

Stimulation and Response. *Distribution of Geotropic Sensitivity.*—In the case of phototropism, a local induction of the stimulus can be obtained easily by screening the remaining regions of the

coleoptile from the light. Geotropic stimulation of the coleoptile locally, however, is an extremely difficult matter owing to the constant action of gravity on all points. The distribution of geotropic sensitivity, therefore, is difficult to determine.

Since the course of the geotropic curvature in the Avena coleoptile begins at the tip and gradually proceeds toward the basal region, Rothert (1894) concluded that the tip is most sensitive to geotropic stimulation, just as it is in the case of phototropic induction. F. Darwin (1899) investigated the distribution of geotropic sensitivity in the coleoptiles of Setaria, Phalaris, and various other grasses by means of a new method: The coleoptile tip was placed in a glass tube and was permanently fixed in a horizontal position. Negative geotropic curvature appeared in the first internode (or in the basal region of the coleoptile) and progressed along the organ, finally producing several coils below the coleoptile (Fig. 50). Darwin concluded from his experiments that a geotropic stimulus was given off constantly from the fixed part of the coleoptile to the lower regions, inducing this portion to curve. The experiments of F. Darwin were not entirely convincing for several reasons, and the problem was investigated further by other workers.

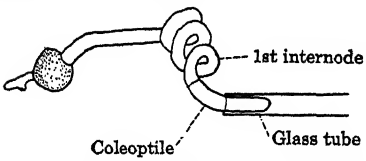


FIG. 50.—Diagram of a *Setaria* seedling, showing coiling response to geotropic stimulation brought about by fixing the coleoptile tip horizontally in a glass tube; after 7 days. (*Adapted from Darwin*, 1899.)

The distribution of geotropic sensitivity in the Avena coleoptile was first described with certainty by employing the method of Piccard (1904). In this technique, the plant is fixed upon a centrifuging apparatus in a sloping position, so that the axis of the apparatus meets the plant organ at an exactly determinable distance from the tip. Then, by centrifuging, the tip and the basal region are influenced in an opposite direction by the centrifugal force, and from the reaction one can estimate the distribution of sensitivity in the plant organ. With this method, Darwin (1908) showed that the curvature appeared in response to stimulation of the coleoptile (*Sorghum* sp.). From this, it was concluded that geotropic sensitivity resided almost exclusively in this organ and that the geotropic stimulus is transmitted from there into the first internode. Thorough investigations on the distribution

of geotropic sensitivity in the coleoptile of *Avena sativa, Hordeum vulgare,* and *Phalaris canariensis* were carried out by von Guttenberg (1912). It was found that a short tip region in these plants (3 mm. in Avena and 4 mm. in Hordeum and Phalaris) is far more sensitive than the lower regions of the coleoptile, though the basal region is not entirely insensitive. Dolk (1929*b*) came to a similar conclusion by another method and found that the length of the sensitive zone in the Avena coleoptile is about 5 mm.

From what has been said, it is clear that geotropic as well as phototropic sensitivity is localized in the tip of the Avena coleoptile. However, there seems to be a difference between the two loci of stimulation, since phototropic sensitivity is mainly confined to the upper 0.5 mm. and geotropic sensitivity to the upper 3 to 5 mm.

Transmission of the Stimulus.—The geotropic stimulus is transmitted from the tip into the basal region just as is the phototropic stimulus. Investigations on the pathway of stimulus conduction were carried out by Boysen Jensen using methods described in the section on transmission of the phototropic stimulus. When a mica plate is inserted into a transverse incision made upon the upper side of a horizontal Avena coleoptile, a strong geotropic curvature results; but when the incision faces downward, the curvature is very weak. From this kind of experiment it can be concluded that the stimulus is transmitted upon the underside of the coleoptile. The problem was investigated also by Purdy (1921); she found that after the stimulation due to wounding had subsided, the size of the negatively geotropic curvature yielded a *d* value of 1.73 mm. when the incision faced upward and a *d* value of 0.74 mm. when the incision faced downward. It is obvious that the stimulus transmission is far stronger on the lower side, though a significant conduction of stimulus can be demonstrated upon the upper side (Fig. 51).

Since the basal region of the grass coleoptile, as well as the tip zone, is affected by gravity, any explanation of curvature that comes about in response to geostimulation of the tip must take into consideration also the direct effects of gravity upon the basal region. Geotropic experiments are probably not so satisfactory as those dealing with phototropism. Zollikofer (1926) showed that transport of growth substance from the coleoptile to the first internode in grass seedlings can be retarded by burning the first

internode. The georesponse of the coleoptile is increased when this is done.

The transmission of a geotropic stimulus is bound up with the transport of growth substance in much the same way as the transmission of a phototropic stimulus. In a horizontally placed coleoptile, the migration of growth substance from the tip

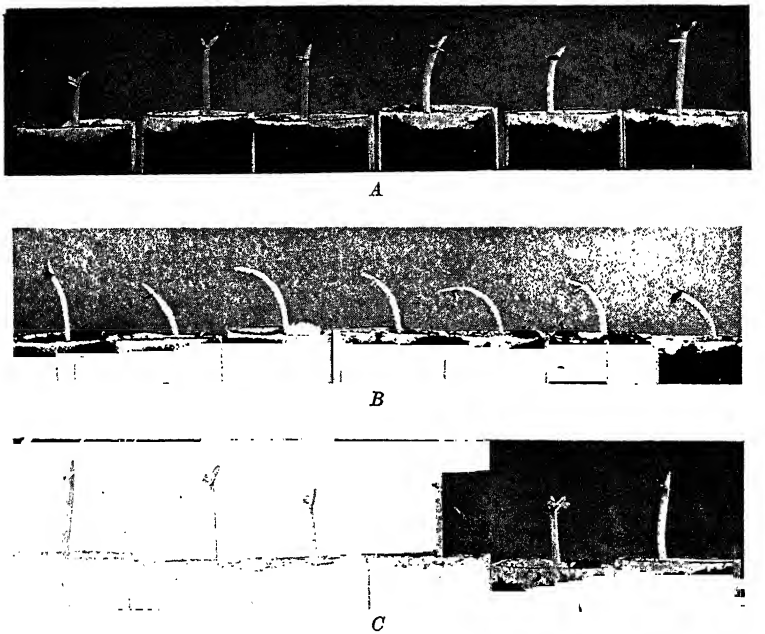


FIG. 51.—Negatively geotropic curvatures in *Avena* coleoptiles. The plants of series *A* and *B* were placed in a horizontal position for geotropic stimulation. *A*, when a mica plate was inserted into a transverse incision made upon the lower side of each coleoptile, only a weak curvature resulted. *B*, when the incision was made upon the upper side, a strong geotropic curvature took place. *C*, control plants. (*After Purdy*, 1921.)

along the underside is increased, while on the upper side it is lessened.

The Quantity-of-stimulus Principle.—Rutten-Pekelharing (1910) demonstrated experimentally that the amount-of-stimulus rule holds for the geotropic curvature of the Avena coleoptile. From Table 11 it may be seen that the product of the centrifugal force and the presentation time is constant within the range of stimulation that has been investigated (in producing a given curvature).

TABLE 11.—THE QUANTITY-OF-STIMULUS PRINCIPLE IN THE CURVATURE OF THE AVENA COLEOPTILE IN RESPONSE TO CENTRIFUGAL FORCE

Presentation time, seconds	Centrifugal force *g*	Product, *g* × seconds
7,800	0.04	312
160	2.24	358
150	2.244	337
120	2.88	346
110	2.907	320
110	3	330
100	3.15	315
90	3.82	334
17	21.66	368
8	40.9	327
7	46.08	322

The Course of Geotropic Curvature.—Before discussing the course of geotropic curvature in detail, it will be well to mention the question of the total growth taking place during the curvature.

With the help of the micropotometric method, Cholodny (1930) compared the rate of growth during the period of geotropic curvature with that of a normal upright-growing Avena coleoptile. He concluded that "all these experiments clearly point to the fact that geoinduction has absolutely no influence upon the growth rate of a coleoptile." It is true that Cholodny's figures for half-hour growth increments fluctuate considerably, and it is probable that small growth changes cannot be demonstrated by this method.

By means of cinematographic photographs, Weber (1931) investigated the course of growth in barley seedlings which lay continually in a horizontal position. Immediately after having been placed in the horizontal position, a difference between the rate of growth of the two sides of the coleoptile became apparent; an increase on the lower side and a decrease on the upper side occurred. The total growth was not demonstrably changed (Fig. 52). When barley seedlings were stimulated by being placed in a horizontal position for 30 minutes, a negative curvature of the coleoptile appeared, followed by several back-and-forth curvatures in the upper zones, where growth promotion on one side alternated with growth retardation on the other.

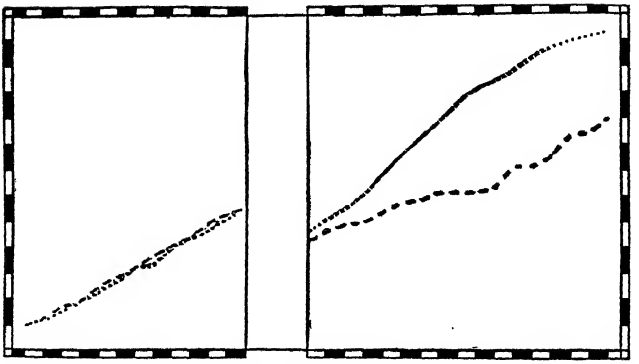


FIG. 52.—Course of geotropic curvature in coleoptiles of *Hordeum* placed permanently in a horizontal position. The dotted line shows the growth of the lower side; the dash line, the upper side. Note the increased growth of the lower side. (*After Weber*, 1931.)

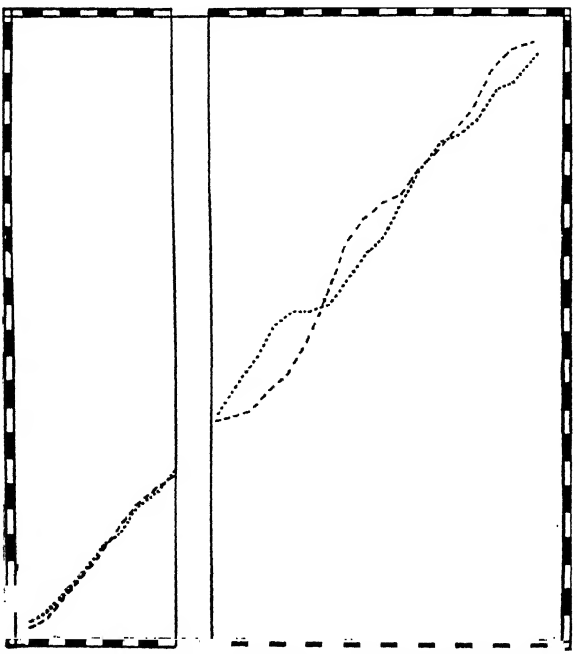


FIG. 53.—Course of geotropic curvature in coleoptiles of *Hordeum* in the first thirty minutes after placing them in a horizontal position. Dotted line shows the growth of the lower side; dash line the growth of the upper side. Note the alternation of the positive and negative growth responses. (*After Weber*, 1931.)

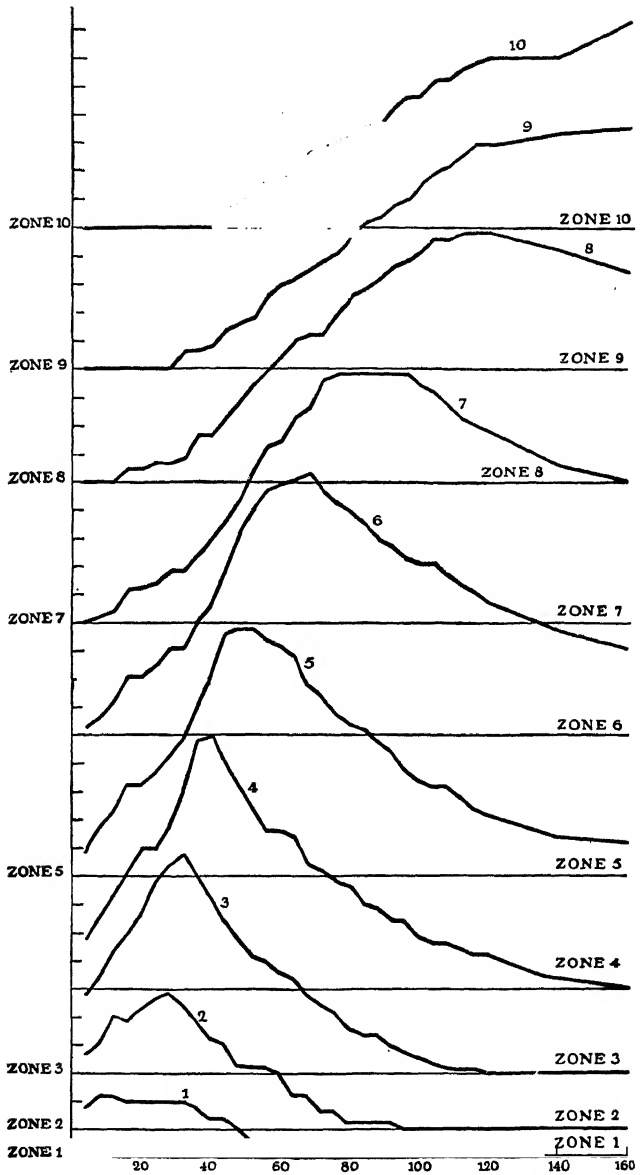


FIG. 54.—Course of curvature in different zones of the *Avena* coleoptile after geotropic stimulation (30 minutes in a horizontal position). Ordinate: the size of curvature (*i.e.*, the reciprocal values of the radii of curvature); abscissa: time in minutes. Zone 1 is at the tip, zone 10 at the base. (*From Dolk*, 1929*b*.)

During these pendulum-like movements, the average rate of growth appeared to be unchanged (Fig. 53).

The course of the geotropic curvature of the Avena coleoptile was investigated by Dolk (1929*b*), who determined the magnitude of curvature in different zones of the organ during response. The seedlings were geotropically stimulated in a horizontal position for 30 minutes, after which they were placed upon the intermittent clinostat in a horizontal position so that the curvatures continued unaffected by the further opposing action of gravity (Fig. 54). Comparison of these curvatures with those resulting from phototropic stimulation (Fig. 46) brings out the following: Curvatures begin in both cases in the tip region, but the geotropic curvature sets in much earlier than the phototropic; in both cases, the curvature proceeds from the tip to the base.

The Growth-substance Explanation. *Growth Substance and Geotropic Sensitivity.*—The suggestion of growth-substance activity in geotropic curvature of the Avena coleoptile goes back to the investigations of Boysen Jensen (1911), who showed that the geotropic stimulus from an excised and replaced tip can be transmitted into the stump. These experiments were confirmed and expanded by Stark (1924), who found that a geotropically stimulated tip, which was transferred to a nonstimulated stump, could cause the latter to curve. These studies showed that a growth substance is concerned in the geotropic curvature of the Avena coleoptile (see Brauner, 1923).

The same problem now arises that was mentioned in the discussion of growth substance in relation to phototropic curvature, *i.e.*, whether only materials involved in normal growth are concerned in geotropic curvature or a special geotropic hormone is formed. The constancy of the rate of growth during geotropic curvature seems to warrant disregarding the latter possibility for explaining negatively geotropic curvature in the Avena coleoptile. By direct measurement, Dolk (1929*a*) demonstrated that the amount of growth substance given off during the curvature is not changed: some coleoptile tips were removed before and some after a geotropic stimulation; both kinds were placed upon agar blocks, and the amount of growth substance given off into the agar blocks was determined in the usual way. The results of the experiments are given in Table 12. Although the figures are not always consistent, it may be concluded that the amount of growth

TABLE 12.—AMOUNT OF GROWTH SUBSTANCE GIVEN OFF BY GEOTROPICALLY STIMULATED AVENA-COLEOPTILE TIPS IN COMPARISON WITH CONTROLS

Time of stimulation, minutes	Number of tips	Time on agar, minutes	Test curvatures, degrees	
			Tips from horizontal coleoptiles	Tips from control coleoptiles
30	8	60	10.3	10.1
33	8	61	8.4	8.4
30	8	60	5.5	6.4
30	8	63	2.2	2.8
30	8	60	7.8	7.9

substance given off from a coleoptile tip is not changed by geotropic stimulation.

The Unequal Distribution of Growth Substance.—Just as phototropic curvature is caused by an unequal distribution of the growth substance present in the coleoptile tip, so geotropic curvature must be also. Dolk (1929*a*) showed that the geotropic stimulus alters the distribution of growth substance in Avena coleoptiles if they are placed in a horizontal position. By placing them in such a position for 30 minutes, then removing the tip and extracting the growth substance from its upper and lower sides, he was able to show that the lower side gives off considerably more than the upper side (Table 13). With a similar technique, Navez and

TABLE 13.—AMOUNT OF GROWTH SUBSTANCE GIVEN OFF FROM THE UPPER AND LOWER SIDES OF GEOTROPICALLY STIMULATED AVENA-COLEOPTILE TIPS

Time of stimulation, seconds	Number of plants	Time on agar, minutes	Test curvatures, degrees	
			Upper side	Lower side
1,800	7	60	4.4	7.0
1,800	8	60	3.0	6.2
1,800	8	60	3.2	4.0
1,800	8	60	2.4	9.0

Robinson (1932*b*) found that a shift in distribution of growth substance in the Avena tip is brought about by change in position of the coleoptile.

Koch (1934) found that when a lateral half of the tip of an Avena coleoptile (1.5 mm. long) is cut away, making the growth-substance supply unilateral, then both geo- and phototropic effects can be compensated for, so that no curvature results (Fig. 55). For example, when the side with the tip present was oriented upward in the horizontally placed coleoptiles, 18 out of 22 showed no negative geotropic bending.

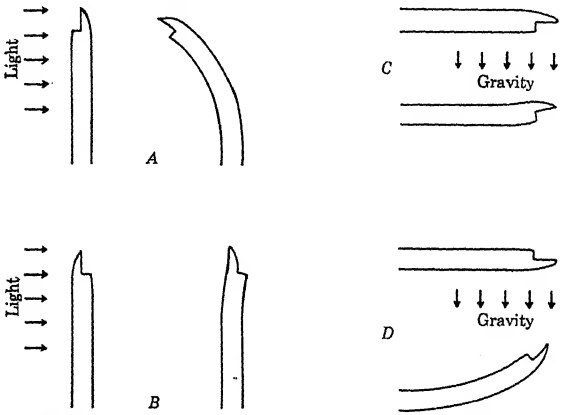


FIG. 55.—Diagrams showing that excision of half portions of the tips of *Avena* coleoptiles affects their tropic response to light and gravity. *A*, when the half tip is removed from the side nearest the light, strong phototropic curvature takes place, due to downward movement of the growth hormone from the half tip which remains on the back side. *B*, when the half tip is cut out from the side away from the light, either no response takes place (due to radially symmetrical distribution of the hormone) or a small negative curvature results, presumably from the slightly greater amount of growth hormone on the side beneath the intact half tip. *C*, growth-hormone displacement due to gravity is diminished when the half-tip portion is removed from the lower side. *D*, negatively geotropic curvature occurs when the half tip is oriented below. (*After Koch*, 1934.)

The problem of how this unequal distribution of growth substance occurs was investigated by Dolk (1929*b*). On the apical end of each of several decapitated Avena coleoptiles was placed a block of agar containing growth substance. The coleoptiles then were placed in a horizontal position, and the growth substance exuding from the basal end was collected separately from the upper and lower sides (Fig. 2). The results (Table 14) indicate that the lower side gives off more growth substance than the upper side. This difference might arise from unilateral changes in permeability due to the action of gravity. According to Nuernbergk (1933), wounding (*e.g.*, by decapitation) retards

TABLE 14.—COMPARISON OF THE AMOUNT OF GROWTH SUBSTANCE RECOVERED SEPARATELY IN TWO AGAR BLOCKS FROM THE UPPER AND LOWER HALVES OF AN AVENA-COLEOPTILE CYLINDER WHICH HAD BEEN SUPPLIED ARTIFICIALLY WITH THE HORMONE AT THE DISTAL END

See Fig. 2 (Dolk)

Time in a horizontal position, minutes	Number of cylinders	Amount of growth substance		Half the original amount of growth substance
		Upper side	Lower side	
120	6	6.3	9.8	11.7
120	6	7.5	10.3	11.7
120	6	4.0	8.8	9.5
120	6	6.5	8.0	9.5
120	5	6.3	8.8	16.3
120	5	3.4	6.8	16.3
120	5	3.8	8.8	16.3

the transverse transport of growth substance ordinarily induced by the unilateral action of gravity. He concluded that transverse transport is brought about by the increased resistance to longitudinal transport on the upper side when the Avena coleoptile is placed in a horizontal position (see also du Buy, 1933). On the other hand, Dolk's work indicates that the unilateral effect of gravity brings about *displacement* of growth substance in the geotropically stimulated tip.

With recognition of the displacement of growth substance in the coleoptile tip as a link in the geotropic stimulus chain, it becomes necessary to examine the separate steps in the process, *i.e.*, how gravity can produce this displacement and how the negatively geotropic curvature arises in the Avena coleoptile as a result of the unequal distribution of growth substance.

The Statolith Theory.—Contemporaneously with certain theoretical observations by Noll (1900), two investigators, Nemec and Haberlandt, suggested that movable grains of starch function as statoliths. If the organ is in a position of geotropic equilibrium, the pressure of the starch grains cannot produce a stimulus. If the organ is taken out of its position of equilibrium, the starch grains accumulate in layers on one side of the cell and exert a pressure upon the plasma membrane of the tangential (and perhaps also radial) walls. This pressure is supposed to produce

a geotropic reaction which results in bringing the organ back into a position of equilibrium.

Nemec (1901) showed that movable starch grains are present in the parenchymatous tissue of the Avena-coleoptile tip. Whether or not they are significant for the perception of the geotropic stimulus was investigated by von Guttenberg (1912), who found that their presence is associated with geotropic sensitivity, although the relationship does not constitute definite proof for the statolith theory.

The literature on this theory has been brought together in an extensive monograph on geotropism by Rawitscher (1932), who came to the following conclusion:

> If we look back over the results of the main experiments, we must admit that a very close relationship seems to exist between the presence of starch and georeception. We must not overlook the role that carbohydrate metabolism plays in the reception of the stimulus of gravity. Whether the starch grains actually function in the sense of the statolith theory as conveyors of pressure has become extremely doubtful in view of more recent observations.

If starch grains actually function as statoliths, it still remains to be explained how their pressure can lead to the unequal distribution of growth substance.

Electrical Theories and Experiments.—Whatever the explanation of the response to gravity may be, the primary reaction is initiated by a traction or pressure effect. According to the electrical theories of geotropic stimulation, differences in potential are produced by the action of gravity upon electrically charged particles which presumably initiate the chain of reactions leading to geotropic response. Small's explanation (1920*a*, *b*) was based upon the colloidal nature of protoplasm in relation to isoelectric points. The assumption was made that the protoplasmic particles in the root are electropositive while those of the stem are electronegative. When the plant organs are placed in a horizontal position, these particles rise to the upper side and in this way bring about radial potential differences. Cholodny (1918, 1923*a*, *b*, *c*, *d*, 1931*b*) assumed that negatively charged microsomes move downward under the influence of gravity. The difference in potential which arises therefrom was supposed to displace the metal ions and lead to a change in the relationship between

univalent and bivalent ions. The unequal distribution of the earth alkalies, which tend to accumulate on the negatively charged side, modifies the swelling properties and the permeability of the protoplasmic membrane and hence, also, the rates of growth. Various objections to these theories can be raised, the main one being that the differences in potential postulated by Small and Cholodny do not agree with those actually found.

In recent years, differences in electrical potential have been described with considerable accuracy in horizontally placed organs (Brauner, 1926, 1927*b*, 1928; Brauner and Amlong, 1933). Brauner discovered the fact that when plant organs are placed in a horizontal position, a difference in potential arises so that the lower side is positively charged with respect to the upper side. This has been observed in both roots and stems. These so-called "geoelectric phenomena" can be demonstrated in dead organs and also in model experiments with parchment-paper membranes. The magnitude of the potential in plant organs fluctuates between 4 and 9 millivolts, according to Brauner; but Amlong (1933) found potentials as high as 34.6 millivolts.

Dolk (1930) and Cholodny (1931*c*) investigated the possible relationship between these phenomena and the transverse displacement of growth substance. Since the latter is an acid, it ought to migrate toward the positively charged pole, *i.e.*, toward the lower side of the root and stem. Although Dolk was unable to discover any movement of growth substance in a potential gradient, several recent investigators have been more successful (Koch, 1934; Ramshorn, 1934; Kögl, 1933, Mitt. VI). The results of experimental investigations on its displacement by gravity are in accord with the findings with respect to electrical potential. The question that remains is whether the potential gradients shown to exist in illuminated or horizontally placed plant organs are adequate to displace growth substance and thus produce a tropic curvature.

In a preliminary work, Brauner and Bünning (1930) reported on studies of the behavior of Avena coleoptiles in an electric field. The experimental objects were placed in a moist chamber and fastened between two aluminum plates so that the lines of force went through the organ. A field of continuous current with a maximum of 640 volts per centimeter was obtained by charging these plates. The coleoptiles curved somewhat weakly toward

the positive pole (Fig. 56). Both the coleoptile of Avena and the root of Vicia were found to be positively charged on the lower side when placed in a horizontal position (Fig. 57). At about the same time, Hartmann (1932) obtained somewhat different results. In experiments with 3,000 volts per centimeter, Avena coleoptiles showed a weak bending toward the negative plate at first and later exhibited curvatures toward the positive pole. These curvatures were always very weak. Amlong (1933) since has studied the electrotropic curvature of Helianthus seedlings according to the method of Letellier, using a field of 1,000 volts per centimeter applied to seedlings growing in a moist chamber. The seedlings curved away from the negatively charged plate.

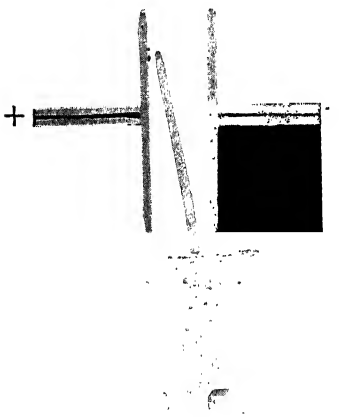


FIG. 56.—Curvature of an *Avena* coleoptile toward the positive pole in an electric field. (*From Brauner and Bünning*, 1930.)

Koch (1934) found that when an electric current is applied through upright coleoptiles placed in conductivity water, the coleoptiles do not conduct the current; hence, within the coleoptile a negative pole is induced on one side, and the coleoptile curves toward it (Fig. 60). When an electric current was applied to roots of Pisum placed horizontally in conductivity water, it was found that they conducted the current and curved to the positive pole, away from the force of gravity (Fig. 60).

A good qualitative agreement has been found between the course of geotropic and electrotropic curvatures, although the former curvature is significantly stronger than the latter. It may be conjectured that the geoelectric phenomenon is probably essential but not the only necessary factor for the displacement of growth substance. It should be remembered that although growth substance may be present, its displacement does not always occur under conditions of unilateral stimulation by light or gravity.

From what has been said it is clear that the first step in the geotropic response in the Avena coleoptile is the displacement of growth substance. The manner of its transport and the changes in rate of growth in the basal region remain to be discussed.

Comparison of Phototropic and Geotropic Curvatures.—As soon as an unequal distribution of growth substance has taken place in the tip of the Avena coleoptile as a result of the action of gravity, a geotropic curvature in the basal region must of neces-

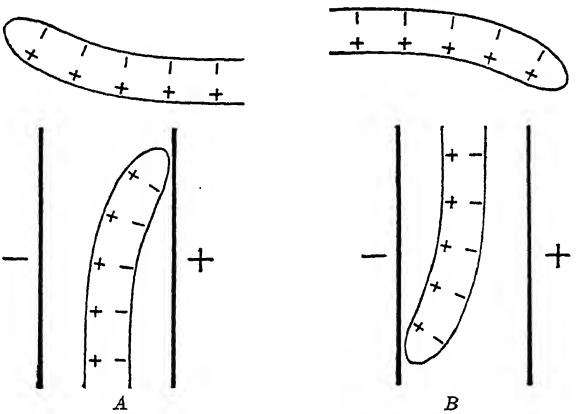


FIG. 57.—Diagram showing distribution of ions in geotropism and electrotropism. *A*, *Avena* coleoptile. *B*, root of *Vicia*. When the plant parts are placed in an electric field, in a moist chamber, an internal polarity results by induction. The coleoptile bends toward the positive pole, while the root bends toward the negative. It is assumed that growth hormone accumulates in the tissue regions which are electropositive, and there promotes growth (in the coleoptile) or inhibits it (in the root). Geotropic response of these organs has been explained on the basis of electrical potentials induced by gravity, as shown at the top of the figure. (*From Brauner and Bünning*, 1930.)

sity result (because of the longitudinal transport of growth substance which follows). From the time displacement of growth substance occurs in phototropism and geotropism, the two types of curvature are essentially the same. There is one difference between the two which might be cited as an objection to the growth-substance explanation, and it may be worth while to examine it more closely (see du Buy and Nuernbergk, 1930). A comparison of the curves (Figs. 46 and 54) showing the course of phototropic and geotropic curvature in the Avena coleoptile indicates that in both cases the curvatures begin in the tip and proceed toward the base; the geotropic curvature sets in much earlier than does the phototropic. Two different conditions may

be pointed out in explanation of this: (1) The phototropic stimulus lasted only 10 seconds in the experiments cited, while the geotropic stimulus lasted 30 minutes. It seems probable that the further steps in the reaction (which take place after the stimulation) probably are beginning during the lengthened period of geotropic stimulation. (2) Since the geotropically sensitive zone is significantly longer than the phototropic, the unequal distribution of growth substance resulting from stimulation may set in more rapidly in the former. In geotropic movement, the curvatures reach a maximum and then decline; in phototropic stimulation, the curvatures of the single zones increase gradually to a maximum and then disappear slowly. In order to explain this difference, it must be recalled that according to the growth-substance explanation, the course of curvature is conditioned by the duration and the magnitude of the unequal distribution of growth substance. Since the first phases of the geotropic and the phototropic stimulus processes are not identical, it is unreasonable to expect a complete agreement in the course of the curvatures. It is not known how long after the cessation of the stimulation the displacement of growth substance continues; possibly the unequal distribution of growth substance is more rapidly equalized when it is produced by gravity than when brought about by light. From the curvatures in Figs. 46 and 54, it can be concluded that a geotropic stimulation for 30 minutes is far stronger than a phototropic stimulation obtained by illumination with 500 meter-candle seconds. This may cause a difference in the course of the curvatures.

Recovery from Geotropic Curvature Brings About Equilibrium.—Dolk (1929*b*) investigated the opposing geotropic curvature (the straightening of the tip) which starts when the rate of growth of the concave side becomes greater than that of the convex. The conditions necessary for the appearance of such a response are that growth substance in the tip be again equally distributed and that an unlocalized supply of it be furnished by the tip to the basal region. With the extraction method, he showed (1930) that more was present in the lower than in the upper side of the tip after the coleoptile had been in a horizontal position for 30 minutes. When the coleoptile was placed again in an upright position, the amount of growth substance extracted from the upper and under side was equal after 60 minutes (see

Table 15). By removing the tip of the Avena coleoptile immediately after geotropic stimulation, Dolk (1929*b*) found that the opposing reaction was greatly retarded. In this way, he demonstrated that a continuous supply of growth substance is necessary

TABLE 15.—COMPARISON OF THE AMOUNTS OF GROWTH SUBSTANCE EXTRACTED FROM THE UPPER AND LOWER HALVES OF AVENA COLEOPTILES WHICH HAD BEEN STIMULATED GEOTROPICALLY AND ALLOWED TO RECOVER

Time in a horizontal position, minutes	Number of tips	Rotation time on the clinostat, minutes	Time on agar, minutes	Amount of growth substance, in degrees curvature	
				Upper side	Lower side
30	6	64	105	5.0	4.7
30	7	60	90	5.8	5.6
30	7	60	90	6.7	7.0
30	7	60	90	6.8	6.6
30	7	60	90	5.3	5.7
30	7	60	90	11.0	10.6
30	7	60	90	12.0	12.5

for the reaction to take place. It appeared after 150 minutes, which is the same length of time required for renewed appearance of growth substance in this region.

During the opposing growth curvature which leads to straightening of the organ, the concave side grows more rapidly than the convex. If one does not assume a shift in the distribution of growth substance, making concentration for a time greater upon the concave than upon the convex side, then one must explain how an equal distribution of growth substance can bring about an unequal rate of growth. Went (1928*a*) had hypothecated a cell-stretching material which, together with growth substance, is necessary for growth. Dolk assumed that more of it is used on the convex side and, therefore, that the concave side where more stretching material remains should grow more rapidly with an equal supply of growth substance. It should be mentioned, too, that the individual cells go through a long period of growth and that the stage of development of the cells also influences the rate of growth of the organ as a whole. On the convex side, the cells may be in a later stage of development than upon the concave

side, and this may be the reason for different rates of growth on the two sides, although the supply of growth substance is equal.

Some observations of a geotropic curvature in the barley coleoptile cannot be explained by the unequal distribution of growth substance (Weber, 1931). When the coleoptile is placed in a horizontal position, a difference in the growth rates of the upper and lower sides arises throughout its whole length. If it is placed in an upright position after it has been geotropically stimulated for 30 minutes, there follow, after the first negative curvature, several positive and negative curvatures which appear to be due to alternating retardation and promotion of the rate of growth in the upper zones. These may be identical with the pendulum-like movements which appear in all negative geotropic curvatures before the position of equilibrium is attained. No curvatures appear in the basal portion of the coleoptile where the vigorous growth is evenly distributed on both sides. Still other cases are known where no growth-substance displacement or geotropic curvature is observed, even though growth substance is present and growth takes place. The young internodes of grasses, for example, behave in this fashion. Further discussion of these cases may be found elsewhere.

DICOTYLEDONOUS HYPOCOTYLS, SHOOTS, ETC.

The main stem of the higher plants is almost always negatively geotropic. Experiments with hypocotyls and stems of dicotyledons have resulted in substantial contributions to our knowledge of the role of growth hormones in geotropism.

Stimulation and Response. *Distribution of Geotropic Sensitivity.*—Satisfactory data on the distribution of geotropic sensitivity in seedling axes have been obtained by Herzog (1925) with the Piccard (1904) method. The following hypocotyls were investigated: *Vicia sativa, Brassica napus, Linum usitatissimum,* and *Lepidium sativum.* All of these were shown to be geotropically sensitive only in the apical portion, and the length of the zone in each species was 11, 18, 16, and 12 mm., respectively. The sensitivity appears to be evenly distributed throughout this region. The ability to respond extends beyond the limits of the sensitive zone, which means that a part of the curvature is induced by a conducted stimulus; the length of this region is about one-third of the entire portion which has the capacity

for curvature. More precise evidence for the transmission of the geotropic stimulus in stems is lacking.

In Bellis, geotropic sensitivity extends over the greater part of the flower stalk but is not uniformly distributed. An apical zone of 7 mm. is more sensitive than the remaining portion.

Negatively Geotropic Curvature in Stems.—Sachs (1872, 1888) measured the upward curvature of various stems in order to discover the course of negatively geotropic curvatures. The zone of growth and curvature of the stem was found to be as long as 15 to 40 cm. At the beginning, the strongest bending was in the vicinity of the tip. As the bend moved downward, the upper originally curved zones straightened; finally, the basal, slowly growing zone often showed a sharp curvature. Sometimes the tip curved back and forth several times before a final position of equilibrium was reached. Sachs showed that negatively geotropic curvatures in stems arise by an increased rate of growth on the lower and an accompanying retardation of growth on the upper side. Sachs did not determine whether the average rate of growth changed during bending. Cholodny (1929*b*), using a micropotometer, concluded that the hypocotyls of Lupinus and Helianthus grow at the same rate, whether in the horizontal or in the erect position.

The Growth-substance Explanation.—In order to show that the geotropic curvature of an organ is produced by the functioning of growth substance, it is necessary to prove (1) that a growth substance is present in the plant organ in question and (2) that the organ responds to the application of the growth substance, the rate of growth being either increased or decreased. If proof of these two points can be obtained, it must be shown, in addition, that gravity brings about an unequal distribution of growth substance. This differential distribution can occur in two different ways: either growth substance may be *displaced* to the lower side as in the Avena coleoptile under the unilateral effect of gravity, or *increased formation* of growth substance may take place upon the lower side. It is possible, also, that the unequal distribution of another substance influences the action of the uniformly present growth substance.

Experiments with Split Stems.—Among the early investigations concerning the role of growth hormones in negatively geotropic curvature of stems, those of Loeb (1916, 1917) should be men-

tioned. In a series of experiments with *Bryophyllum calycinum*, it was found that geotropic growth takes place on the lower side of horizontally suspended stems and that the response is much greater if a leaf is present on the stem (Fig. 1). From his numerous experiments, Loeb postulated the presence of hormones as a plausible explanation for the observed phenomenon.

Gradmann (1925) tried to determine the presence of growth substance during geotropic curvatures in different plants belonging to the Labiatae and Scrophulariaceae. Whole internodes of Mentha and other plants were placed in a horizontal position, and the epidermis was removed from the top and bottom sides. Other internodes were split lengthwise, and the cut surfaces placed in contact with the exposed surfaces of the whole internodes. After a time, the central internode curved toward the upper contact surface. This and other similar experiments led Gradmann to the conclusion that growth-promoting substances are present in the lower half. However, the experiments were complicated by the fact that traumatic effects were introduced by the removal of the epidermis.

The experiments were criticized by Cholodny (1927, 1929*a*, *b*, 1931*c*, *f*; see Gradmann, 1931), but the various hypotheses and auxiliary hypotheses which were propounded scarcely warrant discussion here, especially since no final decision concerning the interpretation of the Gradmann experiments has seemed possible. An improved method would be very desirable for clarifying the situation.

If Gradmann's experiments could be considered as conclusive evidence, it would follow that the growth-substance content of the lower halves is greater than that of the upper halves, even when both are isolated. This difference cannot be attributed to growth-substance displacement. Both halves would react separately during the geotropic reaction, and a harmonic combined effect would not result. It is of fundamental significance to know whether this assumption is actually correct, and from this point of view it might be profitable to study geotropic response in split stems.

When intact barley coleoptiles are placed in the horizontal position, they show an increased growth rate on the lower side and a retarded growth rate on the upper side (Weber, 1926*b*). If the coleoptiles are split lengthwise, an exchange of growth substance

between the upper and lower sides cannot take place, and one would expect that the halves would grow at the same rate at which they do when in a vertical position. According to Weber, this is not the case, for although the lower half grows at approximately the normal rate, growth is retarded in the upper half. In both cases, the growth substances apparently accumulate on the side that is downward. Since the cut surface of the upper half is on the under side, it might be assumed that wounding influences the effectiveness of the growth substance and decreases the rate of growth, whereas no effect of this kind is present in the under half. This agrees with some of Cholodny's (1931*c*) experiments, in which the retarding influence of unilateral wounding upon the rate of growth depended upon how the wound surface was oriented with respect to the direction of gravity. The rate of growth in Helianthus hypocotyls, with the wound on the top, was 1.5 to 2 times as great as that in plants with the wound on the under side.

The circumstances that exist in split dicotyledonous stems should be considered briefly. It is known that a median longitudinal cut retards the rate of growth appreciably (Sachs, 1873; Schtscherback, 1910) and that when a split stem is brought into a horizontal position, the under half grows more rapidly than the upper half (and faster than a control half which is placed in a vertical position). In an investigation of geotropism in many plants, Ahrens (1934) found that when hypocotyls were halved lengthwise, only the lower halves reacted geotropically. The results of these experiments, however, are influenced so greatly by traumatic effects that it is impossible to draw final conclusions about the production of the geotropic curvature.

Loeb (1917) marked portions of Bryophyllum stems into 1 cm. segments with India ink. Each stem portion possessed one or more vigorous leaves and was placed in a horizontal position. After several days, geotropic curvatures took place as a result of stretching growth of the cortex in the lower half. In other experiments, the stem was split lengthwise into two equal halves, each having one vigorous leaf left at the apex. The stem portions were suspended horizontally in moist air, one with the cortex below, the other with the cortex above (Fig. 58). Only that half stem with the cortex oriented downward showed geotropic bending. It may be pointed out that the tissue into

which growth-promoting substances are transported must of necessity be capable of growth response in order to accomplish geotropic bending. In the Bryophyllum half stems, no growth curvature can be produced when the woody tissue and pith are oriented downward. Many variables enter into all these experiments with split stems which possibly vitiate the results, *e.g.*, the errors in halving the stem, the unequal degree of drying of the surfaces, etc.

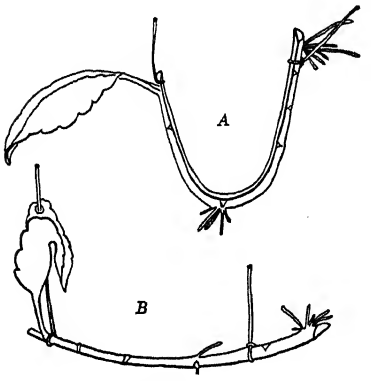


FIG. 58.—*Bryophyllum* stem split longitudinally, each half possessing a leaf at its apex. The split halves were suspended horizontally in moist air. *A*, with the cortex below. *B*, cortex above. Geotropic curvature occurred only in the half stem with the cortex below. (*After Loeb*, 1917.)

Growth-substance Displacement.—Dijkmann (1934) investigated the geotropic curvature of the Lupinus hypocotyl and demonstrated the presence of growth substance there. It was present over the whole growing zone; the growth rate was proportional to its concentration, within certain limits. Growth-substance displacement toward the lower side of a stem placed horizontally was also studied. The results indicated that the lower side contains more growth substance than the upper side, but the results of these experiments are not so clear as those on hypocotyls. If a hypocotyl cylinder placed in a horizontal position is completely covered with growth substance on the apical end, it is possible after a time to extract more growth substance from the lower than from the upper half of the morphologically basal end (Fig. 2) (Dijkmann). From these experiments, it can be concluded that the geotropic curvature of the Lupinus hypocotyl results from displacement of growth-substance to the under side and that the behavior of this organ fits in with the growth-substance explanation.

Van der Laan (1934) investigated the same problem, using the epicotyl of *Vicia Faba*. When it was oriented horizontally, more growth substance could be extracted from the under than from the upper side, an indication that displacement had occurred (Fig. 59). When the seedlings were treated with ethylene, the

greater part of the growth substance disappeared, as did geotropic sensitivity, and the shoots grew horizontally. The small amount that was left was unequally distributed, more being found in the upper half. Since the quantity remaining after treatment with ethylene was very slight, the measurements may not be significant.

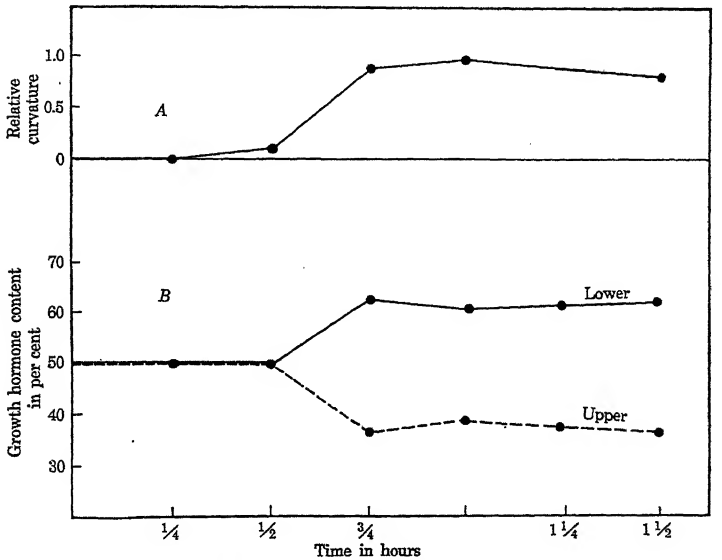


FIG. 59.—Negatively geotropic curvature and differential distribution of growth hormone in horizontally placed seedling stems of *Vicia Faba*. *A*, the course of curvature. *B*, the growth hormone is equally distributed in the upper and lower halves during the first half hour in the horizontal position; in the ensuing 15 minutes there is a shift in the concentration, so that the lower side contains 65 per cent, the upper side, 35 per cent. The higher concentration on the lower side is correlated with the negatively geotropic curvature. (*After Van der Laan*, 1934.)

Boysen Jensen (1936), using the chloroform extraction method, investigated the distribution of growth substance in seedling axes of Phaseolus and Vicia during geotropic curvature. In accord with previous work, he found a greater concentration in the under side than in the upper, although the difference in concentrations was not so great as might have been expected from the observed rates of growth on the two sides.

According to Beyer (1932), geotropic curvatures observed in stalks of Taraxacum, hypocotyls of Impatiens and Cucurbita, and

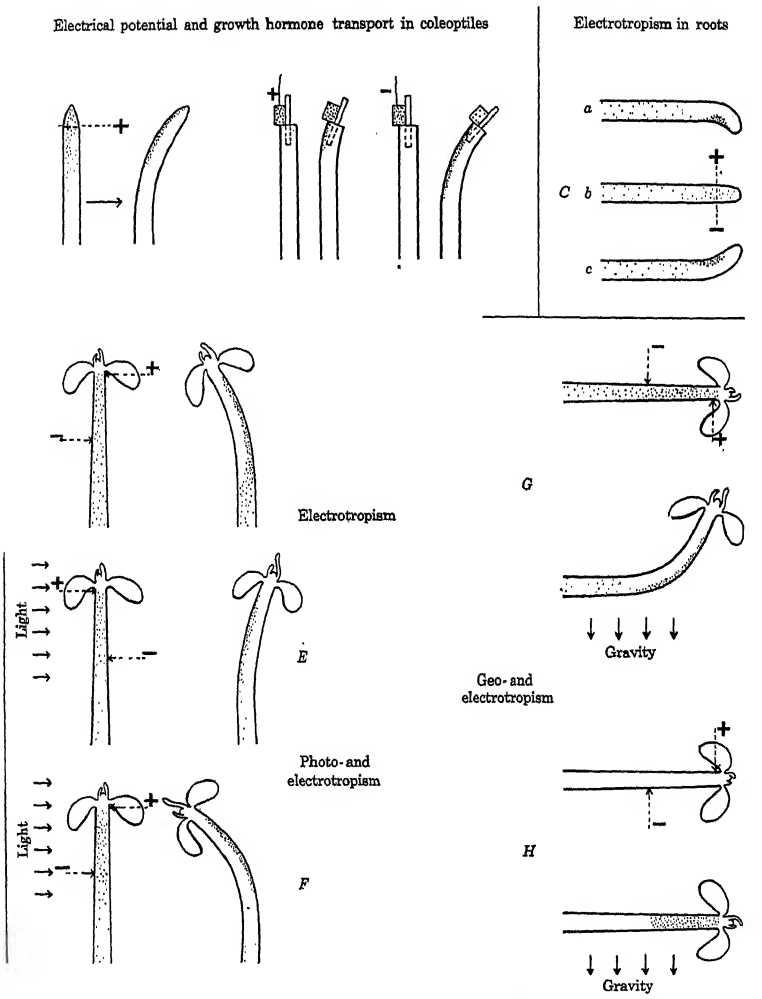


FIG. 60.—Electrical potential in relation to growth curvatures. In accordance with its acid character, the active radical of the growth-hormone molecule bears a negative charge; hence it should be moved toward the positive electrode in an electrical circuit. Accumulated hormones in the relatively electropositive tissues should regulate growth in agreement with the theory of growth-hormone activity. Density of stippling indicates the presence and relative concentration of the growth hormone. The diagrams show the setup and the resulting growth response in each experiment.

A, effect of an electrical field upon growth curvature. Upright coleoptiles immersed in conductivity water bend toward the positive electrode in a circuit. This may be explained on the assumption that the coleoptile with its cuticle

hypocotyls and epicotyls of Helianthus cannot be brought directly into accord with the growth-substance explanation. In Taraxacum, during the course of curvature, growth increased and often was renewed in stalks that had previously ceased elongating. It was shown that these organs may curve geotropically after growth in length has stopped. A shortening of the upper side was reported in Helianthus. Beyer did not investigate growth-substance content or distribution, so it is entirely possible that formation of growth substance could have been renewed in these horizontally placed organs and thus account for the observed revivals of growth.

The investigations of Gundel (1933) showed that in geotropically curved parts of a plant, the acidity upon the convex side is heightened while that of the concave side remains unchanged. Metzner (1934) found increased acidity and plasticity of the cell walls upon the convex side of Helianthus hypocotyls. In accord with the investigations of Strugger (see page 113), this observation might be of significance for the comprehension of geotropic curvatures in seedling stems of dicotyledonous plants. It is not known what influence the accumulation of

is not a conductor, hence by induction the internal polarity is positive on the side toward the negative electrode.

B, electrical polarity and longitudinal transport. Decapitated *Avena* coleoptiles with growth substance applied in agar blocks on the apical cut surface were put in series with a small battery by attaching wet silk threads to the agar blocks and closing the circuit at the other end where the roots dipped into the water of the culture vessel. Transport of the auxin anion into the stump (as determined by the amount of the ensuing curvature) was retarded by making the base of the coleoptile negative to the tip, as shown in *a*, and promoted by having the base positive to the tip, as in *b*. The potential employed was 80 millivolts and the current 0.0008 milliampere.

C, effect of an electrical circuit upon geotropic curvature in roots. The main root of *Pisum* placed in a horizontal position normally curves downward, *a*. When immersed in conductivity water in an electrical circuit with the positive pole above the root, as in *b*, the root conducts the current and bends toward the positive pole, even away from the force of gravity, *c*.

D, effect of applied potential upon erect *Helianthus* hypocotyls; they curve away from the positive electrode.

E and *F*, influence of applied potentials upon phototropism. *E*, when the negative electrode is on the shaded side of a unilaterally illuminated *Helianthus* hypocotyl, the electrotropic stimulus is stronger than the phototropic, and the hypocotyl bends away from the light. *F*, when the shaded side is made positive, normal phototropic curvature is augmented.

G and *H*, influence of applied potentials upon geotropism in hypocotyls of *Helianthus*. *G*, in a horizontally placed hypocotyl, negatively geotropic curvature is augmented by making the lower side positive. *H*, normal geotropic curvature does not occur when the upper side is made positive. (*B after Kögl*, 1933*b*; *all others after Koch*, 1934.)

acids may have on releasing growth substance from an inactive state. (See further discussion on pages 113–115.)

Electrical Transport of Growth Substance.—In this connection, the investigations of Koch (1934) throw some light upon the problem. It was found that the hypocotyls of Helianthus moved toward the positive pole when an artificial potential was applied to them (Fig. 60). The geotropic response was easily compensated for electrotropically by inducing a positive charge on the upper side of the horizontally placed hypocotyl. The negatively geotropic response could be increased considerably by applying the positive lead from a battery to the lower side. The author concluded that displacement of growth substance, in accordance with its acid character, took place in the direction of the positively charged region, with consequent effects upon growth and tropic curvature (Fig. 60).

Along this same line, also, Ramshorn (1934) showed correlations between growth intensity and electrical potential which seem to be in accord with the growth-substance explanation. Brauner (1935) found that characteristic potential differences appeared in all the plant tissues investigated when they were in a horizontal position. The under side becomes electropositive to the upper side, showing maximum potentials of 35 millivolts. This geoelectric effect is independent of the geotropic sensitivity of the organ and its life conditions. Working with suitable membrane models, Brauner and Amlong (1933) suggested a possible origin of these potentials in the influence of gravity upon diffusion potentials (see Brauner, 1927*b*). Brauner's (1927*b*) statement that the geoelectric effect is independent of living processes, while photoelectric potentials arise only in living tissues, points to a marked difference in their origins. Further than this, Brauner (1935) concluded that although electric potential causes growth-substance transport leading to geotropic response, quite a different mechanism (such as the movement of growth substance by modification of cell permeability) is concerned in phototropism.

Inheritance of Geotropic Response.—Comparative studies of geotropism in different species of plants have yielded some interesting information with respect to the possible inheritance of the mechanism of response to gravity. In a study of the geotropic response of Capsicum fruit stalks, Kaiser (1935) found that a single gene difference is responsible for the dominance of

pendant over erect fruit in the F_1 generation. Van Overbeek (1936*a*) studied geotropic response in a variety of maize commonly referred to as "lazy." He reports that when grown in darkness the young plants up to 5 or 6 days old are negatively geotropic, as are normal seedlings; when grown in light, the plants are ageotropic. Brain (1933) observed a seasonal variation in the gravitational irritability of *Lupinus arboreus* and *L. polyphyllus*, while *L. albus* appeared to be equally responsive throughout the year. The first two species were termed "physiologically zygomorphic" because their sensitivity to gravity was greater for the cotyledonary than for the intercotyledonary plane, while the last was equally sensitive for both planes of the hypocotyl. These and other variations between closely related plants seem to indicate clearly that certain tropic properties may be inherited.

Geotropic Response in Nodes.—It is well-known that in many plants (particularly in the grasses) portions of the axis in the region of the nodes remain potentially embryonic long after the rest of the internodal parts have differentiated into mature tissues. When such plants are displaced from their normal position with respect to gravity, negatively geotropic curvatures occur.

The geotropic response of mature grass nodes was investigated by Sachs (1872) and DeVries (1880). When grass stems were placed in a horizontal position, growth in length of the nodes was renewed on the under side, while the upper side became compressed. Two effects of gravity should be distinguished in this case: (1) the resumption of growth and (2) the unilateral distribution of growth. Elfving (1884) made the noteworthy discovery that growth was resumed under the unlocalized action of gravity, attained when the plant was rotated on a clinostat.

As has been mentioned in the chapter on normal growth, Schmitz (1933) demonstrated the presence of growth substance in young growing internodes. In spite of possessing growth substance, the young internodes do not curve geotropically, nor can accumulation of growth substance on one side be demonstrated. Young nodes also contain growth substance, but the mature ones do not. However, when a plant with mature nodes is rotated on a clinostat, formation of growth substance occurs apparently as a result of the unlocalized action of gravity.

Growth substance is formed anew under the unilateral action of gravity, but it is found only on the under side. When geotropically stimulated nodes were excised, split lengthwise, and placed unilaterally upon decapitated Avena coleoptiles, a curvature resulted from use of the under halves only. These experiments were carried out with nodes of Triticum, Secale, Lolium, Holcus, and Setaria. In an experiment using the under halves from 40 test plants, there were 27 negative, 10 straight, and 3 positive curvatures; using the upper halves of nodes from 26 plants, there were 3 negative, 20 straight, and 3 positive responses. A clear difference exists between the growth-substance content of the upper and lower halves of geotropically stimulated nodes.

There are two possibilities concerning the origin of growth substance as a result of the unilateral effect of gravity. Either the growth substance is formed exclusively in the cells of the under half of the node, or else it is formed in all cells and is immediately displaced to the under side as a result of gravity. Schmitz deals with the first possibility in the following words: "Growth substance is formed anew upon the under side of a node when it is unilaterally stimulated by gravity, but it is formed in the whole node when it is subjected to unlocalized stimulation on a clinostat." The second possibility of general formation followed by unilateral displacement is the most probable. The growth and curvature in halved nodes lend strong supporting evidence. As deVries originally showed, the upper as well as the lower half of nodes can curve geotropically which must mean that growth substance is formed in the upper half also, when it is in a horizontal position. The fact that it cannot be demonstrated there in intact nodes can be explained as due to displacement to the lower side.

While the nodes of grasses act independently in geotropic curvature, in other plants, such as Tradescantia, there is some relationship between the curvature of one node and the node next above it. Kohl (1894) showed that the negatively geotropic curvature of a node of Tradescantia could be suppressed by removal of the node above. He concluded that the stimulus is received in the upper node and is transmitted from there to the lower node where the reaction occurs. Miehe (1902) showed that this view was not correct, for, if the internode between the two nodes was bent so that the upper node was vertical and the lower

one horizontal, a curvature still appeared at the lower node. Stimulus reception and response both appear exclusively in the lower node. Schumacher (1923) objects to Miehe's experiments because the vertical position of the upper node is essentially a position of stimulation, since the shoots are plagiotropic. According to investigations by Uyldert (1931), the influence of the upper node upon the lower one is brought about by the giving off of growth substance. Actually, the presence of growth substance was not demonstrated in the organs in question, but when growth and geotropic curvature of the node was checked by removal of the internode, an application of growth substance brought about the usual response to gravity.

ROOTS

Roots are very sensitive to the force of gravity, but not all roots exhibit the same type of response. Primary roots grow toward the earth's center, *i.e.*, they are *positively geotropic*; branch roots frequently grow out at an angle and assume a position more or less transverse to the direction of the earth's force, *i.e.*, they are *diageotropic*. Recent investigations indicate that growth hormones, similar in nature to those occurring in leaves and stems, control in a peculiar way the processes of growth and tropic curvature in roots.

Stimulation and Response. *Distribution of Geotropic Sensitivity.*—In roots of *Vicia Faba*, the geotropic curvature at first is evenly distributed over the 3 mm. at the tip of the root (Sachs, 1882*b*). After 23 hours, the curvature in the extreme tip has disappeared, the region just behind is only slightly bent, and most of the curvature is back about 3 mm. This means that the response is confined to a very short region in contrast to the distribution of the negatively geotropic curvature of the stem.

It was shown by Ciesielski (1872) and Darwin (1881) that the ability of the root to curve geotropically could be checked by removal of the root tip. From this, Darwin concluded that the stimulus is perceived in the tip and transmitted from there to the growing zone where the reaction takes place. Keeble and Nelson (1935) found that amputation of the tip 1 mm. nearly always removed the capacity for geotropic curvature even when the root remained in a horizontal position for 24 hours. That this decapitated root could still perceive the stimulus of gravity was

shown by the fact that when reheaded and placed vertically, the root curved toward the side that had been lowermost when the root was horizontal (Keeble, Nelson, and Snow, 1929). Although it has been shown that the growth of the root is in no way retarded by decapitation, the possibility still exists that the ability of the growing zone to react is lessened by decapitation. It would be far more satisfactory to have the localization of sensitivity in the tip demonstrated by experiments with intact plants, but such a requirement is much harder to fulfill for geotropic than for phototropic stimulation. Czapek (1895) tried to obtain data along this line with the use of his well-known tube experiments. Haberlandt (1908), Jost (1912), and Dewers (1914), using the method of Piccard, showed that if the tip and basal regions of a root were stimulated in opposite directions on a centrifuge, the whole root reacted uniformly in response to the stimulation of the tip, provided the length of this tip was 1.5 to 2 mm. It may be concluded that the tip of the root is more sensitive to the effect of gravity than the proximal regions and that the stimulus must be transmitted from the tip into the zone of reaction.

Stimulus Transmission.—Snow (1923, 1924*a*) gave the first direct proof for transmission of stimulus in the root. He showed that when the root tips of *Vicia Faba* were removed after geotropic stimulation and then replaced on the cut surfaces with gelatin, geotropic curvatures took place. Of 76 roots treated in this way, 45 showed positively geotropic curvatures after 15 to 24 hours, the mean size of the curvature amounting to about 30 deg. Of 32 decapitated roots, on which the tip was not replaced, 27 remained straight, 4 showed a weak positive curvature, and only 1 exhibited a strong curvature. Snow concluded that the geotropic stimulus can be transmitted over a wound surface, and his work was confirmed by Boysen Jensen (1933*c*). In decapitated roots of *Vicia Faba* (variety Windsor White), the *d* value of the geotropic curvature which took place in 24 hours amounted to 0.04 mm. (average of 36 plants); in decapitated roots with replaced tips, it amounted to 0.44 mm. (average of 39 plants).

Snow determined upon which side of the root the stimulus was transmitted. A transverse incision was made either upon the upper or on the under side of roots of *Vicia Faba*, and a mica plate was inserted in the wound in order to check the transmission of the stimulus over the wound. The roots were placed in a hori-

zontal position, and the size of the geotropic curvature was measured in degrees. This value was corrected for the wound curvatures caused by the incision.

The following numbers were obtained:

Incision on the upper side... $(-39.7°) + (-7.0°) = -46.7°$
Incision on the lower side.... $(-27.7°) - (-7.0°) = -20.7°$

Snow concluded that the stimulus can be transmitted on the upper side as well as on the lower side, although greater transmission of the stimulus takes place on the under side of the root.

Keeble and Nelson (1935) showed that although the secretion of a growth substance inhibiting root elongation must be localized in the 1 mm. tip zone of *Zea mays*, the region capable of receiving

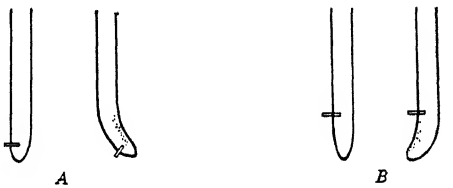


FIG. 61.—Transverse insertion of mica plates into roots of *Zea mays*, and their influence upon resulting curvatures. *A*, when the plate is inserted near the tip, bending occurs away from the wound, due presumably to hindrance of growth-hormone transport from the tip into the elongation region. *B*, if the plate is inserted farther back from the tip, curvature occurs toward the wound, due presumably to the accumulation of hormones in the elongating region. (*After Keeble and Nelson*, 1935.)

the stimulus of gravity extends possibly into the whole elongating region. By inserting small mica plates transversely halfway through the root at different distances from the tip, it was possible to demonstrate that (1) negative curvature takes place when the semisection hinders growth substance from being transported to the elongation region on the cut side and (2) positive curvature (toward the wound) results when the section hinders growth substance from escaping from the region of elongation on the cut side (Fig. 61).

The Quantity-of-stimulus Principle.—A definite amount of stimulus is necessary for the attainment of a minimum reaction in the geotropic curvature of the root. The geotropic stimulus intensity can be changed by altering the direction of perception of gravity. The effect may be computed in this case as g sine φ, where φ is the angle between the root and a vertical axis. Another

method of controlling the stimulus is by the use of centrifuging, which can be accurately graded by changing the rate of revolution or the length of the radius; Pekelharing (1909) investigated the threshold reaction of Lepidium roots (see Table 16) by this

TABLE 16.—THE THRESHOLD VALUE OF CENTRIFUGAL FORCE FOR CURVATURE OF *Lepidium* ROOTS, ILLUSTRATING THE QUANTITY-OF-STIMULUS PRINCIPLE

Presentation time, seconds	Centrifugal force *g*	Product, *g* × seconds
1,260	0.284	358
780	0.44	343
560	0.67	375
315	1.14	359
120	3.15	378
60	6.20	372
30	12.8	384

method. It appears from her data that the product of the presentation time by the force is a rather constant value, within certain limits.

Growth in Geotropic Curvature.—For the production of a minimum curvature, a definite amount of stimulus is necessary. If the amount of stimulus is increased, the size of the reaction is also increased. Lundegardh (1918) made a quantitative study of curvature in the root tip after different amounts of geotropic stimulation (Table 17). The relationship between the increase

TABLE 17.—INCREASE IN AMOUNT OF CURVATURE OF AVENA COLEOPTILES WITH AN INCREASE IN THE AMOUNT OF CENTRIFUGAL FORCE

Amount of Stimulus, *g* Minutes	Curvature, Degrees
5	12
10	22
20	47.8

of curvature with increasing quantity of stimulus is approximately linear. With great amounts of stimulus, a negative instead of a positive curvature takes place in the root (Jost and Stoppel, 1912; Jost and Wissmann, 1924).

It is important to know whether or not the average rate of root growth during geotropic curvature remains constant. Sachs (1887) concluded that the geotropic reactions in seedling roots

"are essentially the same as those involved in the upward curvature of the shoot axis." "During the curvature, a decrease in the rate of elongation appears in the growing axis; while the convex side grows more rapidly, the concave side grows less than would be the case in undisturbed growth in a vertical direction." That seedling roots show no noticeable change in growth rate when they are placed upon the clinostat was shown at about the same time by Schwarz (1881) and Elfving (1883). Later, Luxburg (1905) investigated the same question and found that the individual difference in the experimental plants was too great to make possible a definite decision. It seemed highly probable that the growth increase in the median zone cannot be large. A retardation of growth in relation to the normal took place in 9 out of 13 roots, while in the remaining ones an increase in growth occurred.

More recently Keeble, Nelson, and Snow (1931*a*) have studied the growth rate of maize roots placed in both horizontal and vertical positions. In all experiments, they found that horizontally oriented roots grew considerably more rapidly than vertical ones. These results, however, could not be confirmed by Cholodny (1932*a*). Navez (1933*a*) repeated these experiments and interpreted the results as follows: When excess water was present so that drops were allowed to form at the tips of roots grown in a vertical position, the rate of growth of these roots was retarded. On the other hand, if surplus water was not present, a difference in the rate of growth could not be shown.

The evidence does not permit a definite conclusion regarding the rate of root elongation during the geotropic response.

The Growth-substance Explanation of Root Curvature.—In a series of experiments with *Vicia Faba* roots, Snow (1923) demonstrated that a geotropic curvature can be produced after decapitation and replacement of the tip. This shows that the stimulus can be transmitted over a wound, and it can be concluded that transmission of the stimulus in the root is bound up with the transport of a substance. The geotropic responses of roots have been studied by Cholodny (1924, 1926, 1927, 1928*a*, 1929*a*, 1931*e*, 1933*b*), who made (1924) the interesting discovery that decapitated roots of *Lupinus angustifolius* produced a positively geotropic curvature when coleoptile tips of *Zea mays* were placed on them. The growth rate of the decapitated roots, so treated, was retarded

about 36 per cent. Then Nielsen (1930*a*) showed that rhizopin (3-indole acetic acid) can completely inhibit root growth in *Lupinus albus* and *Vicia Faba* without permanently injuring the roots. Other work by Cholodny (1926) supplied the information that although decapitation has a retarding effect upon the rate of growth of the coleoptile, it has a promoting effect upon the growth of the root. The increase amounted to only about 12 per cent. According to Keeble, Nelson, and Snow (1930), the growth-retarding substance accumulates in the wound when the root is decapitated. When the substance is removed by washing, growth of the decapitated root again takes place. If the excised tip is replaced, the rate of growth is decreased again. All these observations conform with the theory of positively geotropic curvature in the root as propounded by Cholodny (1927). According to Cholodny's theory, a growth substance, which is identical with that formed by the coleoptile tip, is secreted by the root tip. This growth substance has a retarding effect upon the rate of growth of the root. The movement of it is influenced by gravity in such a way that it accumulates upon the lower side of horizontally placed roots. As a result, the rate of growth of the root is retarded on the under side, and a geotropic curvature results.

In 1932, Hawker published some experimental data which pointed to the presence of growth substance in the root of *Vicia Faba*. Geotropically stimulated root tips were cut longitudinally into an upper and lower half. Four such pieces (all either upper or lower) were placed on each of a series of gelatin blocks. After one hour the half tips were removed, and the gelatin blocks were placed unilaterally upon decapitated, vertically suspended *Vicia Faba* roots. Curvatures appeared, due to bending toward the side with the gelatin block. The curvatures were greatest in those roots with gelatin blocks which had obtained something from the lower halves of the original root tips.

In a study of the influence of temperature upon geotropism in seedlings of *Vicia Faba*, Hawker (1933) demonstrated conduction of the geotropic stimulus into the root from excised and replaced root tips. Excised tips from plants grown at 20°C. were found capable of causing greater geotropic bending than tips taken from plants which had grown at the same temperature but had been kept at 5°C. for 24 hours immediately preceding the experiment. A period of 24 hours in the cold apparently decreased the produc-

tion of growth substance in the root tips (Fig. 62). It may be considered, therefore, that displacement of growth substance is responsible for the geotropic curvature in roots.

Similarity of Growth Substance in the Root and Coleoptile.—The experimental evidence shows that the growth substance of the coleoptile has a retarding effect upon the growth of the root;

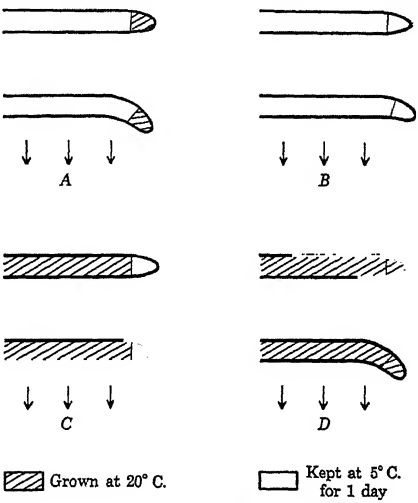


FIG. 62.—Effect of temperature on the geotropism of seedling roots of *Vicia Faba*. The upper member of each pair of diagrams shows the set-up, while the lower one shows the response after 24 hours. The plants were grown at 20° C., but those without cross-hatching were kept at 5° for 24 hours preceding the experiment. The tips were excised and replaced on the stumps in different combinations as follows: *A*, 20° tip on 5° stump; *B*, 5° tip on 5° stump; *C*, 5° tip on 20° stump; *D*, 20° tip on 20° stump. The greatest curvatures occurred in those roots bearing tips which had grown at 20° C., *A* and *D*, due probably to the greater growth-hormone content of the tips. (*After Hawker*, 1933.)

therefore, placement of a coleoptile tip upon a horizontal, decapitated root will produce in it a positively geotropic curvature, since the growth substance accumulates on the under side of the tip. These observations alone are not sufficient for a complete explanation of positively geotropic curvature in the root. It is necessary to show, in addition, that growth substances that have a promoting effect on the growth of the Avena coleoptile are actually formed by the root tip. It must be shown, also, that the increased growth which takes place following removal of the root tip is not produced by wound substances of an entirely different nature (Bünning, 1928).

Cholodny attempted to prove that growth substance is formed by the root. When Avena coleoptiles were decapitated, it was found that the average growth rate and the capacity for phototropic and geotropic curvature were decreased. In 4 to 5 hours after decapitation, the tip is "physiologically regenerated," and the ability to grow and curve in response to stimuli is regained. Knowing that by applying growth substance to the decapitated coleoptile (reheading with the coleoptile tip) the ability to curve can be produced sooner, Cholodny (1928*a*) then tried to show that the same thing happens when root tips instead of coleoptile tips are placed upon decapitated Avena coleoptiles. He also (1929*a*) applied root tips or root cylinders of Zea to decapitated Avena coleoptiles and investigated the rate of growth. A typical experiment consisted of three series of decapitated oat coleoptiles (10 plants in each) selected for comparable length. To one series were added Zea root tips, to another Zea root cylinders, and the third remained as a control. In the first, the average increase in growth was 9.3 per cent in 7 hours at 18°C.; in the second, 7.4 per cent; and in the third 7.2 per cent, under the same conditions. The root tip seems to be more effective, but the amount of increase over that of the controls is not great. It should be pointed out that within the 7-hour period, a "regeneration" of the tip in the Avena coleoptile would have taken place, and this factor may have influenced the results.

Keeble, Nelson, and Snow (1931*b*) investigated the effects upon growth and tropisms produced by placing excised tips of coleoptiles and roots upon decapitated roots. Their experiments indicated that the same kind of growth regulator exists in both roots and coleoptiles, though it inhibits the growth in length of roots and promotes elongation in coleoptiles.

In another series of experiments, Cholodny (1934) tested the growth-promoting effect of excised tips from the roots of *Zea mays* placed unilaterally upon decapitated Avena coleoptiles. In all cases, negative curvatures resulted within 2 hours, and it was concluded that a growth substance was present in the root tips. In other experiments, a root tip was placed on one side and a coleoptile tip on the other side of the Avena stump. Under these conditions, no curvatures took place, or bending was toward the side occupied by the root tip. From these results, he concluded that an isolated tip of a Zea root gives off about the same

amount of growth substance as the excised tip of the Avena coleoptile.

A further clue as to the chemical nature of the growth hormone present in the roots of *Vicia Faba* was obtained by Heyn (1935) from determinations of the coefficient of diffusion of the substance through a series of agar blocks in contact. The coefficient of diffusion was computed to be 0.391 as compared with a similar value of 0.409 for the growth hormone extracted from Avena coleoptiles and a theoretical value of 0.416 for auxin *a*. This would indicate that "the hormone is almost certainly identical with ordinary auxin."

Extraction of Growth Substance from Roots.—Other investigators have obtained data which throw doubt upon the presence of growth substance in the root tip. Bünning (1928) carried out numerous growth measurements on different roots and concluded from his experiments that the stimulus of a wound can have a retarding as well as a promoting effect upon the growth of the root and, furthermore, that the root tip does not give off growth substance. Gorter (1932) came to the same conclusion as a result of experiments designed to demonstrate its presence in root tips of *Zea mays* and Pisum. Tests were made by placing root tips unilaterally upon decapitated Avena coleoptiles or by the method of extraction of growth substance from the root tips into agar blocks or moist sand. It was not possible by these methods to demonstrate that any growth substance was given off by the root tips.

Boysen Jensen (1933*b*) discovered that the extraction of growth substance from the root tip is easily accomplished when 10 per cent dextrose is added to the agar. He thought originally that the sugar in the agar had a plastic nutritive effect upon the root tip. However, the dextrose may be replaced by mannite, which is scarcely detectable in a root tip. The effect might be a physical one, influencing diffusion in some way, though Cholodny (1934) is of the opinion that the nutritive idea is correct, because the excretion of growth substance into nutritive agar is continued for a relatively long time. Further work by van Raalte (1936) using *Vicia Faba* root tips has shown that not only does more auxin diffuse out into the agar made up with 10 per cent glucose, but that there is 3.3 times as much auxin present in the root tips which have been in contact with glucose agar (chloroform extrac-

tion method). These results make it highly probable that the presence of sugar in the agar promotes auxin formation in the root.

Boysen Jensen and others have determined the amount of growth substance contained in the various root zones and found that the amount decreases gradually from the tip backward, being confined mainly to the apical 6 mm. It can be assumed on the same grounds as previously mentioned in the chapter on phototropism that no special geotropic hormone is formed in the root tip.

Transverse Distribution of Growth Substance.—Boysen Jensen (1933*c*) showed that more growth substance is given off into a dextrose-agar block from the under half of a geotropically stimulated root tip than from the upper half. The experiments were carried out in the following way: After seedlings of *Vicia Faba* had been in a horizontal position for 3½ to 4 hours at a temperature of 19°C, the root tips (about 2 to 4 mm.) were cut off with a razor. Blocks of dextrose agar were placed one above the other upon a carrier at a distance of 0.55 mm. from each other (Fig. 2). The root tips were then placed upon these agar blocks in such a way that the upper half rested upon one block and the lower half upon the other block. The root tips and agar were then placed in a saturated atmosphere for 2 to 5 hours after which the tips were removed from the agar. With the help of the marks which are left by the tips upon the agar blocks, it was possible to select blocks on which the tips had been precisely placed. These were moistened with an alcohol-citric acid solution and kept overnight at a low temperature. The following day they were placed unilaterally upon decapitated Avena coleoptiles, and the resulting curvatures were measured. It was found that the blocks that had received growth substance from the under halves of the root tip produced greater curvature than those treated with the upper halves. It may be concluded that more growth substance is given off from the under than from the upper side of a geotropically stimulated root tip. Further experiments with the root tips kept in a horizontal and in a vertical position while the growth substance was extracted yielded a difference between the *d* values in the former instance amounting to 0.40 mm. (27 plants) and, in the second case, 0.32 mm. (37 plants).

Although the unequal distribution of growth substance in the root tip, due to gravity, could come about in various ways, it may

be assumed with a fair degree of certainty that its accumulation on the lower side of a horizontally placed root is the result of its transverse movement.

Mechanism of Growth-substance Displacement.—The geoelectric phenomenon, whereby the lower side of the root is positively charged with respect to the upper, through the action of gravity, may be significant for the transverse displacement of the growth hormone. Whether root curvatures can be brought about by a potential gradient has been investigated with diverse results. Letellier (1899) studied the effect of a zinc plate charged to between 384 and 576 volts upon buried roots of *Vicia Faba*. When the plate was positively charged, a negative curvature resulted; when negatively charged, the curvature was not obvious. In the experiments of Brauner and Bünning (1930) mentioned previously, a strong curvature toward the negative pole resulted in roots of *Vicia Faba* in a field of continuous current of 640 volts per centimeter (Fig. 63). If one assumes that the displacement of growth substance is brought about by a difference in electric potential, it should be expected that this displacement would be in the direction of the induced positive tissue. In the roots of *Pisum sativum*, Hartmann (1932) observed curvatures in the direction opposite to those observed by Letellier, though the same methods were employed. In Amlong's (1933) experiments with roots of *Vicia Faba*, there was a good qualitative and quantitative agreement in the first hour between the course of the geotropic and electrotropic curvature, but later on the geotropic curvature became by far the stronger. In evaluating these investigations one comes to the same conclusion as that which was reached for the Avena coleoptile, *viz.*, that an electrostatically produced potential difference is not sufficient to produce a curvature of the same magnitude as that found in geotropic

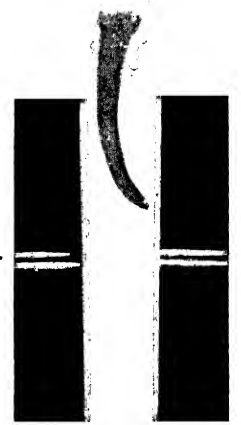


FIG. 63.—Curvature of a *Vicia Faba* root in an electric field. When a root is placed between electrodes in a moist chamber, it bends toward the negative electrode. Compare with Fig. 57. (*From Brauner and Bünning*, 1930.)

curvature. Amlong tried to explain this discrepancy on the assumption that the opposing action of gravity is not excluded in electrotropic curvatures. Future experimental investigations must determine whether this explanation is valid.

Koch (1934) has given some valuable evidence in support of the electrical potential theory of growth-substance displacement in roots. The roots of Pisum were placed horizontally in conductivity water, and an electric current from a small battery was applied to the system. It was found that the roots conducted the current; they curved toward the positive pole (Fig. 60). The same electrical potential applied to wet agar containing growth substance caused movement of the latter to the positive pole. In other experiments where growth substance was applied unilaterally to the tip of a seedling root, Koch found that a bending toward the side of growth-substance application resulted. When he extracted growth substance from Avena coleoptiles and applied it in different concentrations to the opposite sides of the tips of vertical Pisum roots, curvatures resulted which were about equal to the difference between the angles of curvature obtained when these same concentrations were separately applied to just one side of the root.

In seeking an explanation for the positively geotropic curvature of the root, it has been shown that growth substance has a retarding effect upon the growth of the root, although up to the present time this has been proved only for definite growth-substance concentrations. Since the rate of growth in the root is increased by decapitation, it may be concluded that in normal roots, growth is retarded by the growth substance which is present in the root tip. It follows that the rate of growth of the upper and lower sides of the root must be different because of the unilateral accumulation of growth substance. The lower side grows more slowly, and a downward curvature is produced. It is difficult to say whether or not the difference which is found in the amount of growth substance in the upper and lower sides is sufficiently great to produce a normal geotropic curvature. The quantitative relationship between fluctuating growth-substance concentration and root-growth rates and the precise determination of growth-substance distribution in the root have not been investigated with methods entirely free from objection. It is highly probable, however, that the positively geotropic curvature

of the main root is brought about by a downward displacement of growth substance induced by the action of gravity. It is probable, also, that the displacement occurs either exclusively or for the most part in the root tip. As shown by Snow (1923), a decapitated root is still able to produce a positively geotropic curvature, provided the tip is again replaced. If the tip is not replaced, the root cannot curve geotropically, even though some growth substance is present in the root stump.

SUMMARY

It has become clear that geotropic *sensitivity* is localized in the tip of the Avena coleoptile and in the tips of the roots of most plants, while in hypocotyls and shoots it is distributed throughout the growing region. The early investigations indicated that a stimulus, probably some chemical substance, was transmitted from the tip back into the growing region. It was shown later (by the agar-block diffusion method) that the growth hormone becomes unequally distributed when the coleoptile, stem, or root is placed in a horizontal position; more of it is found in the lower than in the upper half. From still other experiments it became clear that the substance in the coleoptile and that in the root are identical.

The manner in which this growth-regulating hormone is displaced unilaterally in an organ subjected to the stimulus of gravity constitutes an important link in the chain of reactions leading to geotropic curvature. When vertically growing organs are placed in a horizontal position, characteristic differences in electrical potential occur; the lower side becomes electropositive to the upper side. Preliminary investigations indicate that this geoelectric effect is independent of the geotropic sensitivity of the organ and the conditions under which it lives. A possible origin of geoelectric potentials has been suggested by experiments with suitable membrane models in which the force of gravity can be shown to have an influence upon diffusion potentials. Geoelectric effects are independent of living processes, whereas photoelectric potentials arise only in living tissues; this must mean that there is a marked difference in their origins. The translocation of growth substance in plants normally takes place toward any region that is electropositive relative to other regions. Artificially applied electrical potentials induce electrotropic

curvatures in Avena coleoptiles, hypocotyls of Helianthus, and roots of Pisum, in agreement with expectations.

Growth substance accumulates in the under side of both roots and stems when they are placed in a horizontal position. The subsequent opposite response of these organs has been pointed to as the explanation of positive and negative geotropism in the two ends of the plant axis. It remains to be shown how the same growth hormone can retard growth in roots, bringing about downward curvature, and promote the rate of growth in shoots, causing upward bending.

CHAPTER X

THE SIGNIFICANCE OF GROWTH SUBSTANCES FOR TRAUMATIC AND THIGMATIC CURVATURES

The curvatures that plants exhibit in response to wounds and other mechanical irritations are well-known, but the causal relationship between the initial stimulus and the final response is not easy to explain. Phototropic and geotropic phenomena bear certain distinct similarities to the traumatic and thigmatic curvatures, for all are the result of differential growth. It seems appropriate, therefore, to examine these wound and touch responses in the light of the growth-substance explanation.

General Survey of the Phenomena.—Traumatotropism of roots was observed first by Darwin (1881) and investigated later by many others. It was found that if a root tip was treated on one side with silver nitrate or wounded otherwise, the resulting traumatic curvature was in a direction away from the wound. Following such unilateral wounding of the tip, transmission of the stimulus takes place, and bending occurs in the growing zone at some little distance away. The growing zone itself is also sensitive to wounding, though to a lesser degree than the tip.

Haberlandt (1921, 1922) postulated the presence of wound hormones to account for the stimulation of cell division at or near the regions of injury in plants (see also Wehnelt, 1927). The observed facts which prompted this hypothesis probably belong to a different category from that which includes traumatic curvature phenomena. Traumatic sensitivity, as we know from the experiments of Stark (1917, 1919), is widely distributed in aerial organs, in the stems of seedlings, and in older stems and leaves.

The thigmatic stimulus first studied in tendrils is rather widely distributed in etiolated seedlings, older stems, influorescences, petioles, etc. (Stark, 1916). Curvatures can be obtained in these plant parts by stroking one surface with a cork rod, whereupon the stimulated side becomes concave in due course of time, and a positive curvature takes place.

Only the most important types of traumatic response will be mentioned, following the general presentation of Stark (1917).

Amputations.—If one cotyledon is removed from seedlings of Helianthus, Sinapis, and many other plants, a positive traumatic curvature appears in the hypocotyl. Positive curvatures occur also, following the removal of part of the leaf lamina (*e.g.*, in Ficaria). After the unilateral removal of leaflets from a compound leaf (*e.g.*, in members of the rose family), curvatures commonly appear in the petiole. When entire leaves are removed (*e.g.*, in *Sonchus palustris*), the axis of the shoot frequently shows a positive, traumatic curvature.

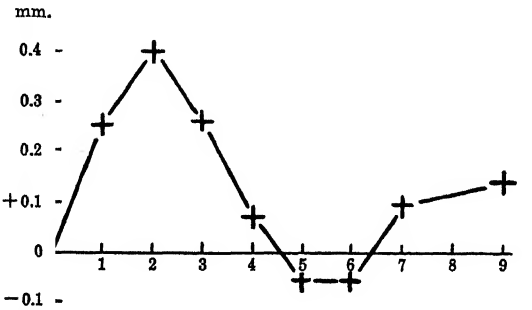


FIG. 64.—Course of a wound curvature produced by a transverse incision near the base of an *Avena* coleoptile. An initial positive curvature is followed by a negative and a second positive curvature. Ordinate: *d* value; abscissa: time in hours. (*After Purdy*, 1921.)

Wounding by Incisions.—Transverse incisions bring about positive traumatic bending in coleoptiles. For example, when an incision is made near the base of an Avena coleoptile, a curvature appears above the wound. Positive wound curvatures can be observed also in hypocotyls, (*e.g.*, of Phaseolus). Purdy (1921) studied the course of curvature in the Avena coleoptile and reported that the initial positive curvature is followed by a negative and a second positive curvature (Fig. 64).

Longitudinal grooves bring about small, positive, traumatic curvatures in Helianthus, Lupinus, Phaseolus, and various grasses. This type of curvature was observed by Boysen Jensen and Nielsen (1925). Later, Weimann (1929) found that a secondary negative curvature appears in both normal and decapitated Avena coleoptiles, whether rotated on a clinostat or not.

Chemical Treatment.—Positive traumatic curvatures can be produced in roots and stems by applying silver nitrate. The effects of certain dyes upon the role of growth substance and upon the normal course of tropic curvatures (Boas, 1933; Schweighart, 1935; Blum and Scott, 1933) have been discussed in previous chapters. Observations on the influence of certain fungicidal preparations are of interest. Neukirchen (1930) treated grass seeds with a series of commercial chemicals and investigated the effect that they exerted upon the geotropic responses of the coleoptiles. Dilute solutions of "Uspulun-Universal" and arsenious acid produced an increase in geotropic response, Uspulun-Universal and mercuric chloride were without effect, and "germisan," formalin, copper sulphate, and eosin caused a retardation of the response to gravity. It is notable that phototropism was not affected by preliminary treatment of the seeds with these same chemicals. Von Witsch (1934) found that very dilute solutions (1/100,000 or 1/800,000 molecular in distilled water) of the salts of heavy metals (copper, silver, uranium) caused retardation of growth and negatively geotropic curvatures in lateral roots of *Phaseolus multiflorus.* The addition of small amounts of calcium or potassium distinctly reduced the growth-retarding effects of the heavy metals without changing the curving effect. Certain other experiments of Warner (1931) indicate that the influence of metal ions upon geotropic response may be due to the effect upon cell permeability. The bearing of such observations upon the growth-substance explanation of tropisms is not known. It may be said that while the traumatic reaction of the root is generally negative, the wound response of coleoptiles and stems generally takes the form of a positive curvature. Meesters (1936) found that chemotropic curvature of the root hairs of Agrostemma (growing in culture solution) did not occur when various concentrations of 3-indole acetic acid, contained in glass tubes, were introduced near the roots.

Stimulus Transmission.—Studies on the transmission of stimuli from the place of wounding to other regions where the response occurs have thrown light upon the nature of the wound stimulus as well as upon the mode of its conduction.

Transmission in the Avena Coleoptile.—In the Avena coleoptile, Stark (1921*b*) showed that both traumatic and thigmatic stimuli could be conducted over an incision. If the tip was removed and

then was replaced, traumatic or thigmatic stimulation of the tip induced a response below the wound. In addition, such a stimulus was transmitted when the coleoptile tip was placed on the decapitated stump of another individual of the same species or even of another species or another genus. The size of the reaction, however, decreased more and more with the distance of systematic relationship of the plants used.

Nielsen (1924), using the Avena coleoptile, made a transverse incision and, after the ensuing traumatic curvature had disappeared, inserted a platinum plate in the wound. The tip was stimulated then, by treating it on one side with silver nitrate, above the incision. If it was stimulated on the side opposite the transverse incision, a positive curvature developed in the basal region; however, when the stimulus and incision were upon the same side, no curvature resulted. This result was just the opposite of that found for phototropic stimulus transmission in relation to incisions.

Transmission in Roots.—Fitting (1907) investigated the path of stimulus conduction in wounded roots and found that it is not hindered by a transverse incision made on the wounded side. Similar experiments were carried out by Ivanovskaja (1929) on the transmission of the traumatic stimulus in the root of *Lupinus albus*. Transverse incisions were made 2 mm. from the tip, and mica plates were inserted. Then the root was stimulated by sticking on one side of the tip a small piece of filter paper, moistened with 0.03 to 0.05 *M* uranyl acetate solution ($UO_2(C_2H_3O_2)_2$). It was found that the negative chemotropic curvature was four times greater when the incision was placed on the chemotropically stimulated side than when it was elsewhere. This means that in these experiments the stimulus was transmitted on the unstimulated side. Later (1930), it was found that the chemotropic stimulus could be transmitted from a root tip that was cut off and replaced on the stump. In addition, it was shown that the stimulus was not intraspecific but that a reaction resulted when a decapitated tip was placed on the root stump of a plant from a different species, genus, or family.

Transmission in Mimosa.—Much work has been done with the transmission of stimuli in "sensitive plants" because of their specialized means of response. It is beyond the scope of this discussion to mention more than a few of these investigations.

Ricca (1916, 1926) observed that a stimulus caused by burning the lower part of the stem can be conducted to the younger leaves through a killed zone or even through water in a short tube. An extract from stem tissue was shown to bring about closure of the leaflets when the petiole of a detached leaf was dipped in the solution. From these experiments, Ricca concluded that the response in Mimosa must be due to a stimulating substance. Movement of the stimulus through the transpiration stream and other rapid means of conduction have been postulated on the basis of experimental data by Snow (1924*b*, 1925*b*, 1925*c*), Dixon (1925), Ball (1927), Bose and Das (1925), and others. Linsbauer (1908) and Umrath (1925*a*, 1925*b*) found that the rapidity of stimulus conduction varies with the intensity and the type of stimulation and the parts of the plant concerned. Carrying forward these investigations with a technique proposed by Bose (1926), Umrath (1928, 1929) was able to demonstrate several rates of conduction by measuring the electrical changes accompanying excitation.

The conclusions of the majority of previous investigators are summarized, and the results of new experiments are presented, in the recent paper of Houwink (1935). This author studied the transfer of stimuli in Mimosa as evidenced by outward movements of leaves and internal fluctuations of electrical potential. For example, stimulation without wounding by the application of a drop of water at less than 10°C. brought about an excitation which was conducted by the living cells. The rate of conduction of the "action currents" depended upon the temperature. Conduction did not occur through a killed part of the petiole or through a zone cooled to 5°C. Wounding or burning led to the formation of a stimulating substance which caused a change in potential. This substance passed through dead zones and through parts of the plant that did not produce it. It was taken in from a wound by the negative pressure in the vessels and transported in the transpiration stream. Excitation brought about by cutting a leaflet could be conducted (1) by action of the cells ("action currents"), (2) by the transport of the wound substance, and (3) by a very rapid mechanism of conduction affecting only the main pulvinus. This last phenomenon was not accompanied by changes in electrical potential and was not conducted through a killed zone but passed through a cooled portion of the petiole.

The Growth-substance Explanation.—From the premises of the growth-substance explanation, traumatic curvatures can be explained in different ways. The supply of growth substance to growing regions might be checked unilaterally, either by destruction of the tissue where it is formed or by removal of the growth substance forming organs or parts of organs. Disturbance in correlated growth activity and curvatures could be the result of inhibition of stimulus transport on one side of the organ. There is the possibility, also, that wound substances are formed (Bünning, 1927) as a result of stimulation and that these substances influence growth. In addition, it may be necessary to consider the possibility that unilateral disturbances in the food supply to growing plant parts can produce curvatures. In view of these different circumstances, an entirely unified explanation of traumatic curvatures cannot be given. The discussion will be confined to the description of a few characteristic traumatic responses, relating them to the possible role of growth substances.

Amputations.—Van Overbeek (1933) showed that the growth substance necessary for growth of the hypocotyl in Raphanus is supplied by the cotyledons. The early report of Heidmann (1913) that the removal of one cotyledon produces a positive traumatic curvature may now be interpreted as being due to the removal of the growth-substance supply on one side.

Wounding by Incisions—When a root or coleoptile is traumatically stimulated on one side near the tip, the resulting curvature of the root is negative, while that of the coleoptile is positive. It is probable that these curvatures arise because of injury to the tip; the distribution of growth substance from the tip is disturbed and is lessened on one side. Lateral incisions in the Avena coleoptile can modify curvatures by interfering with the transport of growth substance. It is more difficult to explain the occurrences in the root. Here a wound below a transverse incision produces a strong negative curvature. This phenomenon might be the result of changes in the concentration of growth substance or the formation of it on the side opposite the wound.

The traumatic curvatures appearing as a result of transverse incisions in the Avena coleoptile have been discussed. The primary positive curvature is explainable as a disturbance of correlated activity, which is brought about by checking the transport of growth substance on one side. The traumatic

curvatures caused by transverse incisions in roots are negative. These curvatures may result from the unilateral inhibition of growth-substance transport, which leads to a negative reaction, since it checks growth in roots. In his discussion of wounding and growth, Cholodny (1931*c*, *f*) showed how wounding can modify phototropic and geotropic responses through effects upon the distribution and activity of growth substance.

Positive and negative traumatic curvatures resulting from wounding a root have been explained in terms of the gradient of growth substance between opposite sides of this organ (Fig. 61) (Keeble and Nelson, 1935). It has been shown that the sap which exudes following the removal of a root tip tends to inhibit growth. Keeble and Nelson found that growth in length is promoted when this exudate is washed away. In the light of other work, it is probable that this root-inhibiting substance in the sap is identical with the growth-promoting substance in the tip of the Avena coleoptile (Cholodny, 1933*b*).

The positive traumatic curvatures which appear as the result of longitudinal incisions can be explained neither by a change in the production of growth substance nor its distribution. A median longitudinal incision in stem organs causes a strong decrease in the rate of growth, but how this occurs has not been determined. Many investigators have conjectured that wound substances are produced as a result of stimulation and that these substances have an effect upon the rate of growth. The fact that Weimann (1929) observed secondary negative curvatures in Avena coleoptiles with longitudinal incisions led him to conclude that growth-retarding wound substances do not exist. Results found in Avena obviously cannot be applied to all plants. Up to the present, it has not been proved that wound substances exist. The retardation of growth by longitudinal incisions may be due to the destruction, as a result of the wounding, of the growth substances normally present. In the instances where growth is promoted by wounding, the effects might be explained on the basis of the freeing of growth substances already present in an inactive form.

SUMMARY

The traumatic and thigmatic curvatures which occur following wounding and mechanical stimulation are brought about by

differential growth, tissue tensions, etc. Some of the factors contributing to such curvatures are changes in electrical potential, modification of physiological processes concerned in growth, *e.g.*, translocation, permeability, etc., and hormones.

From the point of view of the hormone explanation of tropisms, wound curvatures may be explained in any one of several ways: (1) The source of the hormone may be removed by excision or injury to the tissues from which it is distributed; (2) impaired transport of the growth regulator (*e.g.*, by a lateral incision) may disturb growth on one side; (3) wound substances may be produced which in turn influence growth. In view of the meager evidence, an entirely satisfactory explanation of these curvatures cannot be given.

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